The systematics and genetics of tomatoes on the Galápagos Islands (*Solanum*, Solanaceae)

By

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Declaration

I, Sarah Darwin confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Chapter 2 is a reprint from a paper on the taxonomy of the tomatoes of the Galápagos Islands published in *Systematics and Biodiversity* in 2003. This was a collaborative project, and the authors were Sarah Darwin, Sandra Knapp and Iris Peralta. I was the lead author as this was part of my thesis work, and I carried out most of the work towards the paper. Below I list the contributions of each author for Chapter 2.

Morphological analysis

I undertook the analysis of the herbarium specimens, with particular guidance from Sandy Knapp and Iris Peralta for the *S. lycopersicum* and *S. pimpinellifolium* collected from the mainland of South America.

Morphometrics

Morphological characters were selected by all of us based on my experience from fieldwork, Dr Peralta's experience from greenhouse grown accessions and Dr Knapp's experience from herbarium specimens. I undertook the measurement of the living plants and herbarium specimens with the assistance/guidance of Drs Peralta and Knapp

Statistics

I and Dr Peralta undertook the PCA, with advice from Drs Claudio Galmarini and Clive Moncrieff.

Taxonomic treatment

Dr Knapp and Dr Norman Robson wrote the Latin for the taxonomic treatment.

Artwork

The illustration of *S. galapagense* for the taxonomic treatment was undertaken by C. Scoones the rest of the illustrations and figures were undertaken by me and formatted for the paper by Derek Adams and myself.

Writing

I wrote the first draft of the paper, and it went through repeated editorial cycles among all three authors.

Sarah Darwin	Prof. James Mallet
Candidate	UCL Supervisor

Abstract

Galápagos tomatoes (*Solanum* section Lycopersicon) have long interested scientists and plant breeders. Several morphological and physiological characters found in the endemic Galápagos tomatoes have been bred into the cultivated *Solanum lycopersicum*, making the native plants an invaluable resource for the development of this important global crop.

Extensive fieldwork was carried out on twelve islands, old records were confirmed, new records added, but several previously recorded populations of endemic tomatoes could not be confirmed. I collected tomatoes from 12 islets and Islands. Detailed morphometric analysis on c.400 plants and extensive genetic studies on c.1,200 plants were carried out in addition to fieldwork and natural history studies. Observations and experiments were carried out on herbarium specimens, field collected and green house grown accessions. I confirmed the presence of four taxa in the Galápagos Islands – two endemic species *S. cheesmaniae* and *S. galapagense*, the latter here described as new, and two introduced species *S. pimpinellifolium* and *S. lycopersicum*.

Hybrids were found involving all taxa growing in the Galápagos Islands. Of particular interest is an extensive hybrid zone between *S. cheesmaniae* and *S. pimpinellifolium* spanning 20km along the Baltra Road on Isla Santa Cruz. Hybridization raises the threat of extinction by introgression and the possibility of the evolution of a serious invasive species. Conservation recommendations are proposed.

In addition, a genetic analysis was undertaken of 60 seed bank accessions of all four tomato species from the Galápagos. Again, indications of hybridization were found, raising the potential of introgression within the seed bank collections, occurring potentially during rejuvenation cycles.

Furthermore, I propose a new outline for a generic invasive species threat/impact scoring system. Applying this new system I class *Solanum pimpinellifolium* as an alien invasive species and assess its impact on Isla Santa Cruz in the Galápagos Islands.

Acknowledgments

I would like to thank my supervisors Dr Sandy Knapp and Professors Jim Mallet and Ziheng Yang for their support and advice during this thesis.

I would like to thank all the many people in the Galápagos Islands for their enthusiasm, invaluable support, advice and help with fieldwork, they are too numerous to mention by name. The Galápagos National Parks, in Islas Santa Cruz and Isabela, for advice, field assistance and field permits. The staff at the Charles Darwin Research Station in the Galápagos Islands, in particular Alan Tye and Mark Gardener for their collaboration and on the ground support. Tina and Daniel Fitter and Virginia Appleton for joining me and guiding me on field excursions. Godfrey Merlin, Jenny and David Day, Felipe Cruz for their general advice and assistance in the Galápagos Islands.

I would like to thank the Tomato Genetic Resource Center and Cornell seed bank for their kind donation of seed accessions.

Clive Moncrieff, Clem Maidment, Iris Peralta, Claudio Galmarini, Steve Ansell, Harald Schneider and Kanchon Dasmahapatra for their advice on analytical packages and statistics and the Department of Botany at the Natural History Museum for giving me desk space on which to undertake this research and in particular my friends Juliet Brodie, Gabrielle To and Rhina and Holger Thüs for their general support. Teamwork in the allozyme laboratory was supported by Johannes Vogel, Steve Russell, Michael Grundmann, Steve Ansell and Harriet Hunt. Pat Hart and David McCann who helped with the maps and layout of figure 3.11. Lorraine Porch who assisted with the thesis layout.

Financial support was received from the BBSRC (PhD grant) and the Hubbard Foundation. The latter funded the first part of the research and the field trips.

Special thanks goes to my family, wonderful husband Johannes, my lovely sons Leo and Josiah, my parents Ras and Shuna for their continued belief and patience and to my mother-in-law Edith Vogel.

I would like to dedicate this thesis to my mother who made our lives such fun.

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Preface

The last decades have seen an increasing interest in the endemic Galápagos tomatoes, not only because they are a fascinating part of the islands' endemic flora but also their potential use in crop improvement. Galápagos tomatoes cross breed freely with cultivated tomatoes and produce fertile offspring (Rick, 1979). Being a close relative of such an important crop plant such as the tomato has meant the two endemic Galápagos species - *Solanum cheesmaniae* and *S. galapagense* - have been extensively investigated for useful characteristics to enhance cultivated tomatoes. For example *S. cheesmaniae* plants from a single population at Puerto Ayora (Isla Santa Cruz) possess an unusual pedicel morphology lacking the central articulation common to potatoes and tomatoes, a mutation known as 'jointless' (Rick, 1967). This allows the fruit to abscise at the base of the calyx which, when bred into the cultivated tomato, has made mechanised picking of tomatoes possible (Rick, 1967). *Solanum galapagense* has been found to be more salt tolerant than more common cultivated tomatoes (Rush and Epstein, 1981). This allowed strains of drought resistant tomatoes to be developed and the cultivation of tomatoes in areas with high soil salinity.

Out of a total of 13 tomato species (*Solanum* section *Lycopersicon* see Peralta *et al*., 2008), only four are edible, thus the two endemic Galápagos taxa represent half the number of edible tomato species in the world. The extensive tomato breeding programmes in the past have used Galápagos tomatoes and the use of tomatoes in many dishes as part of our everyday diet and the use of tomatoes in many dishes means that we are feasting regularly on germplasm of these endemic species.

My personal interest in Galápagos tomatoes dates back some 15 years. I first read about the Galápagos tomatoes while visiting the islands during 1995/6 to undertake fieldwork before illustrating a field guide to the flora of the Galápagos Islands. One of my tasks was to illustrate the endemic tomato species then called '*L. cheesmanii* forma *typicum'* and '*L. cheesmanii* forma *minor'*. A red-fruited tomato species was at the time widespread along the roadsides in Puerto Ayora (Isla Santa Cruz) and towards the highlands of Bella Vista (to the north of Puerto Ayora). The fruit was popular and commonly consumed by local

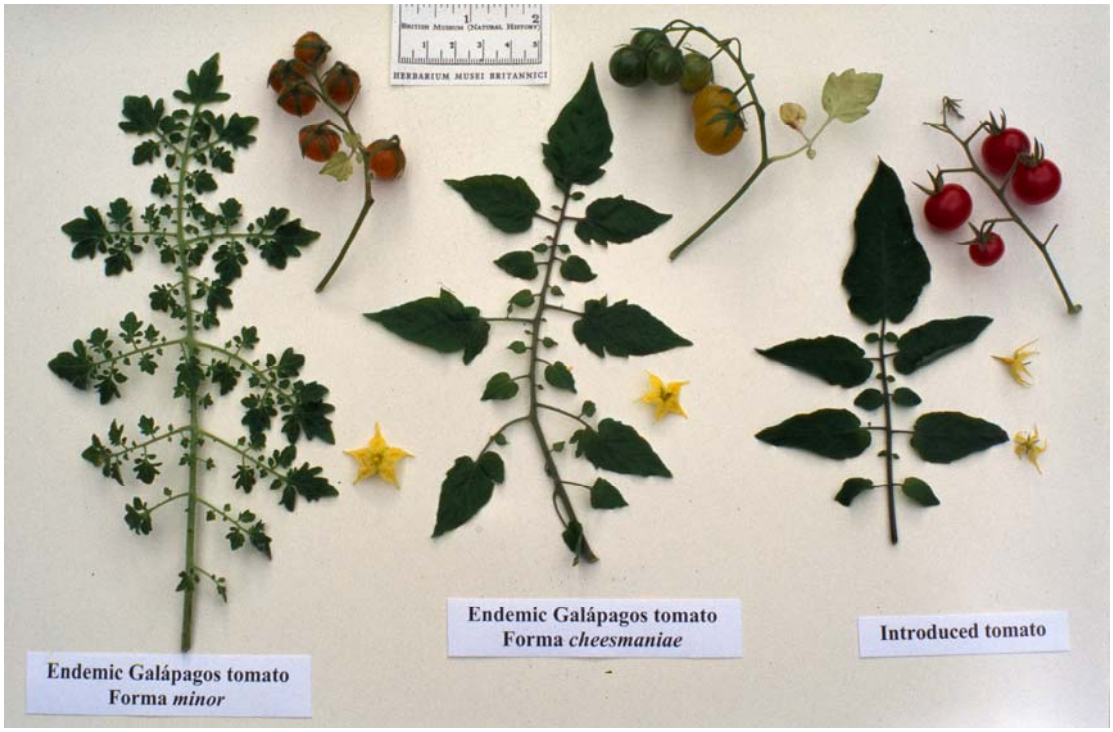
residents. This plant was then widely described by locals on Isla Santa Cruz as “the Galápagos tomato” and it also appeared in several field guides to the islands identified as the endemic tomato (e.g. Jackson, 1983; McMullen, 1999). However, studying the treatment of *Lycopersicon* in the Flora of the Galápagos Islands (Rick in Wiggins and Porter, 1971) revealed that the native tomato taxa had yellow to orange fruit. There was thus an anomaly here between public/widespread perception of what constituted the Galápagos tomato and botanists described as the endemic tomato.

When I returned to the UK, I wrote to various Galápagos and Solanaceae experts from all over the world. I was eventually directed to the late Professor Charles Rick from UC Davis California, who was the director of the Tomato Genetic Resource Center (TGRC). Professor Rick had collected and studied tomatoes in the Galápagos Islands since the 1950s. Through these travels he had acquired over 60 wild accessions of tomatoes from the Galápagos Islands and these were incorporated in the TGRC seed bank collections. In correspondence he confirmed that the endemic Galápagos tomatoes indeed had yellow to orange coloured fruit and he informed me that any red fruited tomatoes in the Galápagos Islands would be *Lycopersicon esculentum* var. *cerasiforme* or *L. pimpinellifolium* (now known as *Solanum lycopersicum* and *S. pimpinellifolium*, respectively). I also contacted Dr Sandy Knapp from the Natural History Museum in London (NHM) who is a world-renowned expert in Solanaceae. She supported the idea that a thorough study on the Galápagos tomatoes would be a very interesting project and was keen to help me pursue to my interests in this subject.

Several years later, in 2000, Dr Knapp contacted me with the exciting news that she had secured funding for a research assistant at the NHM (from the Hubbard Foundation) to study Galápagos tomatoes. Meanwhile I had graduated with a BSc in botany (1999) from the University of Reading and thus was able to start working at the Department of Botany at the NHM in April 2000. Later in 2000 my post mutated into a BBSRC funded PhD studentship at University College London under the supervision of Dr Sandy Knapp and Professors Jim Mallet and Ziheng Yang.

In order to establish the morphological variation of the different species of tomatoes in the Galápagos Islands we obtained loans from different herbaria from around the world enabling me to examined large numbers of specimens which had been collected from the Galápagos Islands and the adjacent mainland of South America. In addition to this I started to grow several plants from six seed bank accessions kindly sent to me from Cornell Seed Bank, which represented the two endemic taxa from the Galápagos Islands as well as accessions of the red-fruited tomato, collected from the islands in 1990s. The three different taxa – the two endemic taxa and the red fruited tomato can be seen in Fig a. All plants were cultivated in the pyramid green house in the Chelsea Physic Garden (CPG) in London.

Figure a. Greenhouse grown plants. Perceived wisdom in 2000; this represents the three Galápagos tomato taxa that were recognised before embarking on Galápagos field work in 2000.



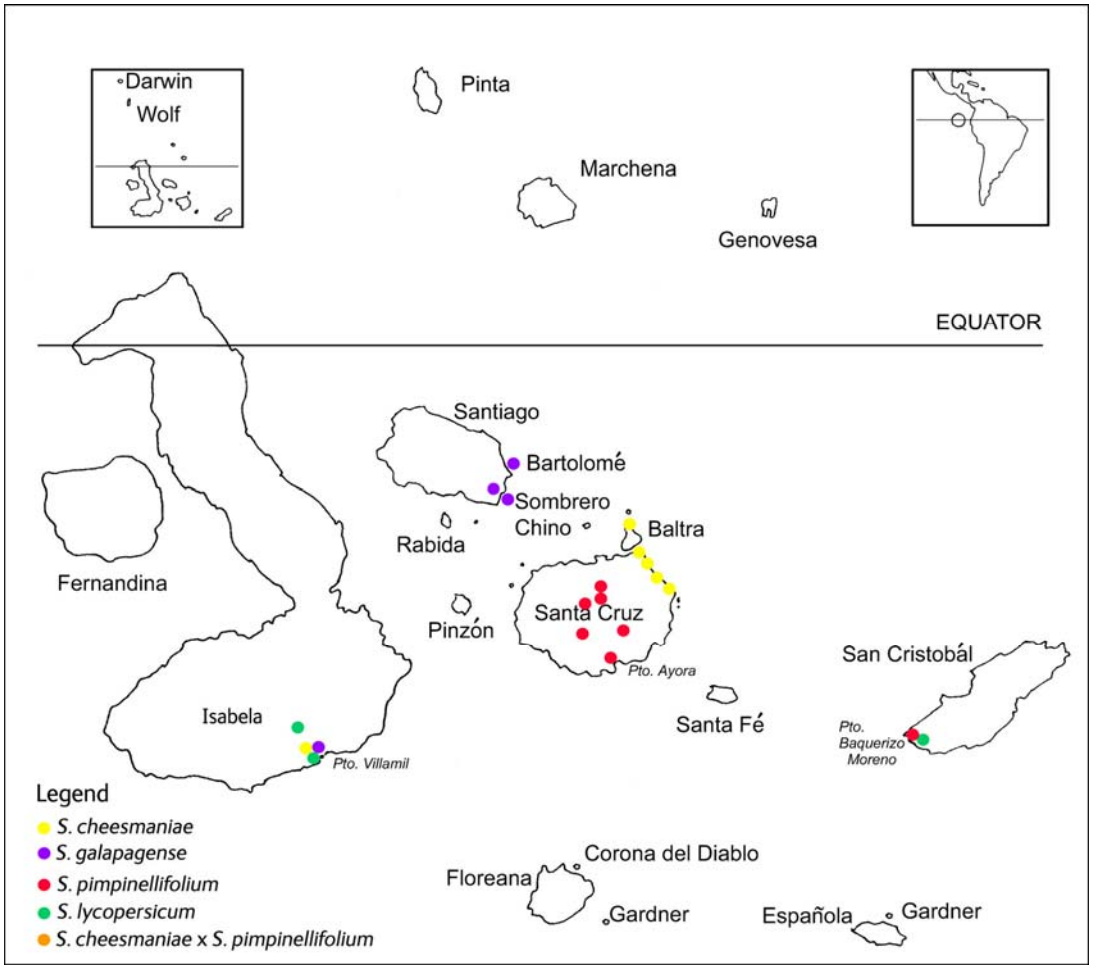
I also started detailed literature search to establish the species circumscriptions within the different taxonomic studies that included Galápagos tomatoes and this turned out to be rather complicated. Joseph D. Hooker (1847) had first published the enumeration of Galápagos plants and this initial research included a list of collection dates and localities of tomato collections since the first Galápagos tomato collections were made in the early 1800s by John Scouler in 1827 (who collected one tomato taxon) and then Charles Darwin in

1835 who collected both endemic tomato taxa. Subsequently, we found an even earlier specimen of *S. galapagense* collected by Archibald Menzies in 1779 from Isla Isabela.

Armed with the knowledge derived from literature and herbarium studies I undertook fieldwork in the Galápagos Islands from July to September 2000. Here, I collaborated with the research station in Puerto Ayora - Charles Darwin Research Station (CDRS) on Isla Santa Cruz. Isla Santa Cruz is the most heavily populated island in the archipelago. As well as CDRS the main Parque Nacional Galápagos office and various NGOs are located in Puerto Ayora. It is a very busy tourist town with many hotels and most visitors to the Galápagos spend at least a few hours in Puerto Ayora. During my stay I undertook various joint field trips with CDRS to several different Islands as well as organizing my own field excursions.

I collected tomatoes from many locations during this field trip – including both endemic and introduced taxa (Fig. b).

Figure b. The Galápagos Islands. SCD field collection sites from 2000



On Isla Santa Cruz I made collections from Puerto Ayora, Bella Vista, El Chato Tortoise Reserve, Los Gemelos, the newly formed rubbish dump (hereafter known as the Basura) and a gravel mine (Mina Roja) as well as along the roadsides between all these localities. In these areas the only tomato I found was the red-fruited tomato plants which were widespread in all these sites. At El Chato the red-fruited tomatoes were so dense that they excluded all other plants. I collected along the north coast of the Santa Cruz but here I only found endemic tomatoes east (and not west) of Punta Carrion.

Solanum galapagense was collected from the south east tip of Isla Santiago. Populations were also found on two small islets off the south east coast of Isla Santiago, Islotes Sombrero Chino and Bartolomé. On Sombrero Chino I found numerous small tomato plants. Tomatoes and the endemic lava cactus

(*Brachycereus nesioticus*) were some of the very few plant species I found on this hat-shaped lava islet.

Then I flew to Puerto Villamil on the south coast of Isla Isabela, which is the largest island of the archipelago, being some 120km long. There are two small villages – the coastal Puerto Villamil and San Tomás which about 10km to the north of Puerto Villamil - on the south side of the Volcán Sierra Negra. The main inhabitants of this island are fishermen but there is also a small farming community at San Tomás. On Isla Isabela I collected *S. cheesmaniae*, *S. galapagense* at a man-made gravel pit (the extract having been used to make the small airport runway) at El Lagoon de Manzanilla. In San Tomás I made collections of both feral and cultivated plants of *S. lycopersicum*. An experimental programme was underway in San Tomás in 2000 to establish which crop plants could be successfully grown in the region (including a cultivar of tomato - *S. lycopersicum* 'Shady Lady'). However, if crops are grown in Galápagos there is a risk of escapees and in order to grow crops natural habitats had to be removed. On the other hand the traffic and trade resulting from the need to import food bring with them the risk of introducing potentially invasive/ dangerous organisms.

Other trips included searches of the coast around Puerto Villamil looking for populations of endemic tomatoes but I only found feral *S. lycopersicum*; I also tried to follow up sites and collections noted by Rick during his active fieldwork in the archipelago (see TGRC website). Rick mentioned some 'ponds' that were just inland from the coast with populations of the endemic tomatoes nearby, but these ponds could not be relocated. Local scientist suggested that there had been a change in the local hydrology of the area due to the spread of a native grass species. I also visited a small island called Isla Tortuga off the coast near Puerto Villamil, but although no tomatoes were found we did make new botanical records for this islet and added several species to its floristic list.

Next I travelled to Isla San Cristóbal, which is another of the five inhabited islands. This island has one of the national airport and is the civic centre of the archipelago. Here, Charles Darwin (in 1835) collected two specimens of tomatoes later described by Hooker (1847) as *L. peruvianum* var. *parviflorum*

and *L. pimpinellifolium* (these specimens now both identified as *S. cheesmaniae)*. Records showed that collections had been made to the south of Puerto Baquerizo Moreno and to the north west of the island. I looked for *S. cheesmaniae* which is recorded in this island but only found *S. pimpinellifolium* and *S. lycopersicum* and plants that appeared to be morphologically intermediate between *S. pimpinellifolium* and *S. lycopersicum* , suggesting that they might be hybrids.

Finally I travelled to Isla Baltra. This island is just to the north of Isla Santa Cruz and houses the Ecuadorian naval base and the larger of the two national airports. Towards the north of this island on the rocky cliffs I found several plants of *S. cheesmaniae*.

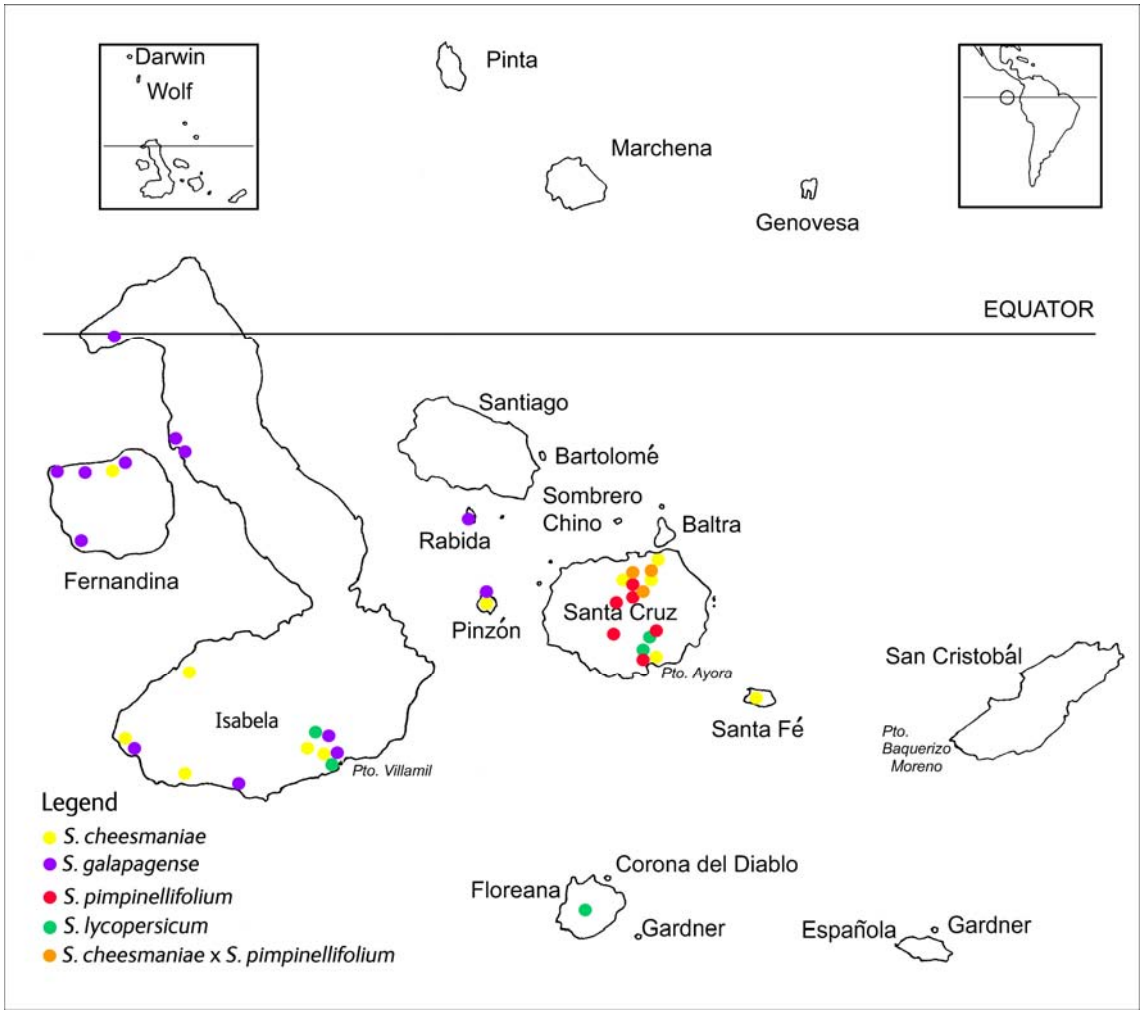
In September 2000 I returned to London with over 340 seed accessions and herbarium specimens. These wild collected seed accessions as well as 54 TGRC seed accessions were grown up into plants at CPG. I had decided that I would analyse the genetics of the plants using allozyme electrophoresis (having undertaken a pilot test run before leaving for the field work) and fresh plant material is required for this technique. The journey from the Galápagos Islands to London at that time took two days. This meant that bringing fresh material from the Galápagos Islands to the UK was logistically difficult, but material from CPG could easily be used for allozyme electrophoresis.

Immediately I started to compare my herbarium specimens with the herbarium specimens that were at BM and that we had on loan from other herbaria. Both, herbarium specimens and greenhouse-grown plants were then analysed morphologically and I use morphometric techniques for a comprehensive taxonomic study. This resulted in the taxonomic paper on Galápagos tomatoes that I co-authored with Sandy Knapp and Iris Peralta (Darwin *et al*., 2003). In this paper we established that four species of tomatoes occur in the Galápagos Islands - two yellow to orange-fruited endemic species - *S. cheesmaniae* and *S. galapagense*, the latter described as a new species and thus an upgrade from having been described as a forma previously at various intraspecific ranks. There were also two introduced taxa with red fruit – *S. lycopersicum,* the cultivated and occasionally feral species of tomato, and *S. pimpinellifolium* a

species with smaller red fruits, which turned out to be an invasive in areas on Isla Santa Cruz. Using my extensive fieldwork, TGRC seed accession and herbarium records enabled we were able to map and determine the distribution of all taxa in the archipelago. The paper was submitted in August 2002.

In September 2002 I returned to the Galápagos Islands to undertake further fieldwork (Figure c).

Figure c. The Galápagos Islands. SCD field collection sites from 2002



The first field excursion was to the Basura on the Baltra Road on Isla Santa Cruz. Here once again I found the invasive *S. pimpinellifolium* growing on the top of the earth mounds at the Basura, just as I had two years before (Figure d).

Figure d. *Solanum pimpinellifolium* at the Basura, Baltra Road, Isla Santa Cruz

Photo courtesy of Daniel Fitter 2002

However this time I also found plants that I recognised as *S. cheesmaniae,* growing in the mesic scrubland surrounding the Basura (Figure e).

Figure e. *Solanum cheesmaniae* at the Basura, Baltra Road, Isla Santa Cruz

Photo courtesy of Daniel Fitter 2002

In addition to the endemic and introduced species, I found plants that were morphologically intermediate between *S. cheesmaniae* and *S. pimpinellifolium* (Figure f).

Figure f. *Solanum cheesmaniae* x *S. pimpinellifolium* at the Basura, Baltra Road, Isla Santa Cruz

Photo courtesy of Daniel Fitter 2002

The fruit had a deep orange colour and other intermediate characters and they were growing on the side of the earth mounds down towards the native scrubland. This was very interesting as these plants appeared to be hybrids between the endemic taxon and the invasive tomato species.

While, from a scientific point of view, finding hybrids between a local endemic and an introduced taxon in such an important plant as tomatoes in the Galápagos Islands is very exciting, from a conservation point of view, taking the biology and natural history of tomatoes (and humans as their dispersal agents)

into consideration, this is probably close to a ‘nightmare scenario’ as far as the future of the endemic Galápagos tomatoes is concerned.

A thorough search of the Baltra Road area revealed several more populations with interesting morphology. These were almost continuous along the roadside for 20km and at the two gravel mines – Mina Roja and Mina Negra. Populations towards the north coast towards the Canal de Itabaca (the Canal) in the lowland arid region were represented by *S. cheesmaniae* and the putative *S. cheesmaniae* x S. *pimpinellifolium* hybrid. However, populations to the south of the Basura where elevations are higher (up to 600m) and habitats are therefore moister (due to precipitation) *S. pimpinellifolium* occurred together with putative *S. cheesmaniae* x *S. pimpinellifolium* hybrids. It is noteworthy that only at the Basura did both taxa and their putative hybrids grow in sympatry. I then collected a transect along the Baltra Road from Los Gemelos at 600m altitude to the coast at the Canal at 5m altitude. Over this transect I made herbarium collections, measured 21 morphological characters on 123 individual plants, collected leaves for subsequent measuring and collected seeds when I found ripe fruits.

Another interesting discovery on Isla Santa Cruz was *S. cheesmaniae* from the Puerto Ayora area. Plants with the ‘jointless’ character were originally collected from populations around Puerto Ayora area. These were the only population of this species that have been found with this mutation where the lack of an articulated pedicel results in the fruit abscising at the calyx. At the CDRS offices I was directed to a single patch of this unique population at the back of their entomology building. This was the only specimen of this important population that has recently been found in Puerto Ayora, despite exhaustive searches. Nuez *et al*. (2004, undertaking fieldwork in 2000) tried to locate but failed to find this population. The presence of individual plants of *S. pimpinellifolium* within a few hundred meters from this important population of *S. cheesmaniae* is obviously of great concern.

Further fieldwork was undertaken to other islands in the Galápagos. Returning to the Puerto Villamil on Isla Isabela to El Lagoon de Manzanilla I made further collections of *S. cheesmaniae* and *S. galapagense* which I found growing in

sympatry. I also discovered individual plants that were intermediate between the two endemic taxa. Furthermore, I was able to make close and extended observations of the endemic carpenter bees (*Xylocopa darwini*) visiting and buzz pollinating both taxa. Thus, I saw how this bee could act as an agent of hybridisation between tomatoes on the archipelago.

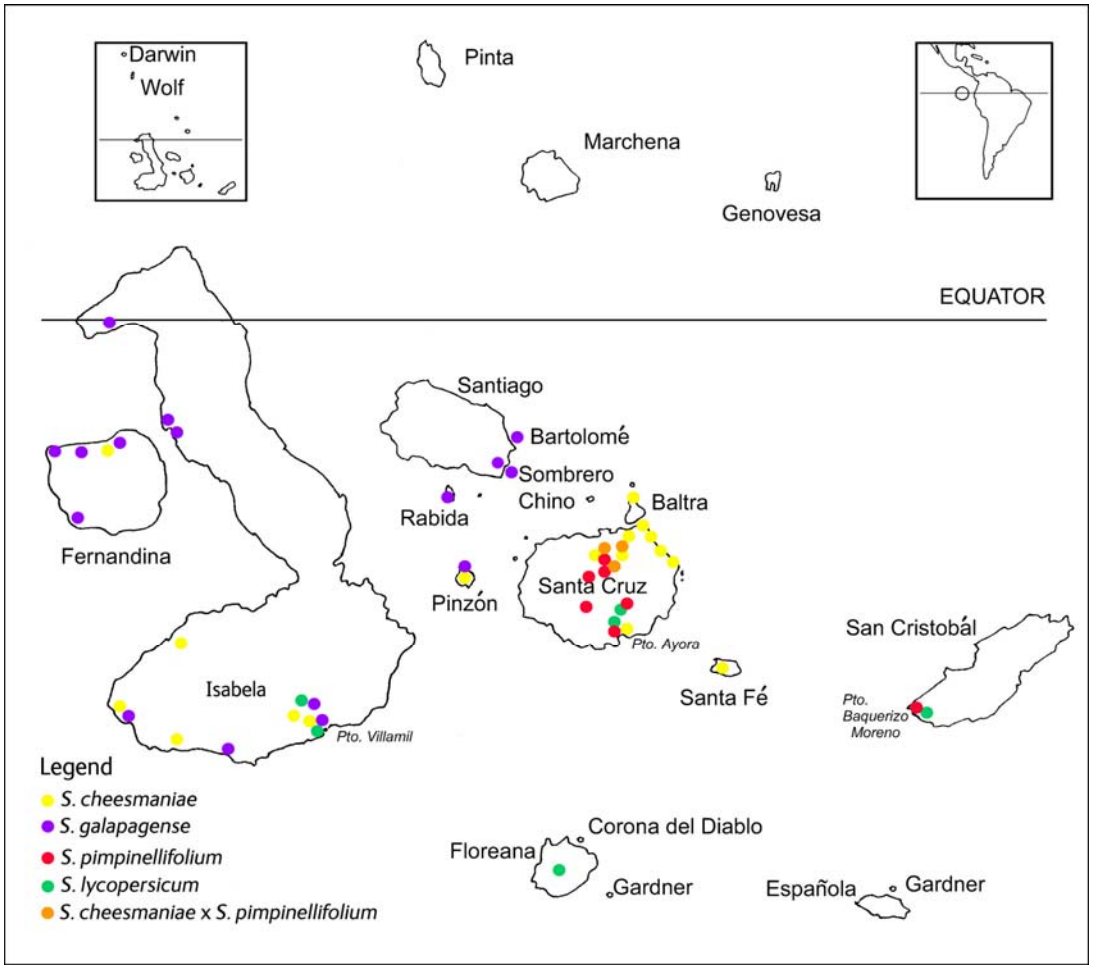
Further field excursions were undertaken to some uninhabited areas. On Isla Santa Fé I only three individual plants of *S. cheesmaniae* but during a trip to Isla Pinzón I collected *S. cheesmaniae* growing at the bottom of the extinct crater and a few individuals of *S. galapagense* from north coast.

In addition to these shorter excursions, I also undertook a ten-day field trip on a boat from Isla Santa Cruz around the south coast of Isla Isabela with members of the CDRS. Rounding the southwest tip of the island we travelled up the west coast towards Isla Fernandina. We collected coastal populations on both Isla Isabela and Isla Fernandina. Following information gleaned from the literature and herbarium sheets our collecting efforts revealed that some previously known populations on Isla Isabela of both *S. cheesmaniae* and *S. galapagense* could not be relocated and my suspicion is that they may be extinct due to goat predation and in some cases recent volcanic activity. However several flourishing populations of *S. cheesmanaie* and *S. galapagense* were collected on Isla Fernandina. At Cabo Douglas on Isla Fernandina towards the northwest point of the island a population of *S. galapagense* was found growing in a lava field with the lava cactus. At Los Túneles to the west of Punta Espinoza I collected *S. cheesmaniae* and *S. galapagense* in sympatry. Sympatric populations of these two endemic taxa were only found on Islas Fernandina and Isabela. Also on Isla Fernandina a collection was made on my behalf from the southwest side of the islands at Cabo Hammond. We continued towards the northern part of Isla Isabela collecting *S. galapagense* on the equator by Volcán Ecuador. We stopped at James Bay, Isla Santiago where we looked for coastal populations of *S. galapagense*. We had also aimed to reach the summit (900m) of Isla Santiago as there was some anecdotal evidence of a red-fruited tomatoes growing at high altitude, but failed to reach this part in the time available due to the tough field conditions of an overgrown walking track with spiny trees and shrubs. We then returned to Isla Santa Cruz. Two other

collections were made on my behalf – *S. lycopersicum* from Isla Floreana and *S. galapagense* from Isla Rábida.

In 2002 I returned back to London with a further 400 specimens, seed collections and morphological measurements. In addition to this, I did manage to bring back 10 fresh leaves from wild tomatoes from the hybrid zone which I collected on my way to the airport. Due to my extensive field work in 2000 and 2002 I was able to confirm the presents of Galápagos tomatoes on 12 islands and islets (figure g).

Figure g. The Galápagos Islands. SCD field collection sites from 2000 and 2002



Further greenhouse plants were grown from the 2002 accessions. The aim was to grow several plants of each of the newly collected accessions for allozyme electrophoresis and also to grow mature plants from the Baltra Road hybrid zone of all taxa and in addition representative plants from pure localities. The

aim was to study a comprehensive set of accessions for further morphometric analysis from samples grown under standardised conditions in the greenhouse environment. These hybrid population plants started flowering in summer 2003; however unfortunately very few *S. cheesmaniae* or *S. cheesmaniae* x *S. pimpinellifolium* putative hybrids produced ripe fruit, while the *S. pimpinellifolium* appeared to cope much better. The 2003 summer was very hot and in hindsight I believe that the humidity may have been too high in the Pit Greenhouse at CPG for *S. cheesmaniae* and a comprehensive comparative data set could not be established. Therefore, I decided to use my field data set for subsequent analyses.

In Chapter 1, I explore the importance of invasive species in general, and on islands in particular. It became apparent to me, due to my long-standing interest in invasive species and especially when studying the literature for this research, that the terminology surrounding invasive species is confused, often contradictory and thus impedes general understanding of invasive species. Part of this chapter examines terminology and suggests practical solutions. This is an important part of the background into which the subsequent detailed studies of introduced tomatoes are set.

In Chapter 2, I (together with Sandra Knapp and Iris Peralta) detail the history, taxonomy and systematics of the four species of tomato on the Galápagos. This is based on my field observations, my own herbarium collections as well as herbarium specimens on loan including all relevant type specimens. A morphometric analysis was undertaken on a selection of herbarium specimens as well as on my own greenhouse grown accessions from wild collected seeds and seed bank accessions. I described *S. galapagense* as new and classified *S. pimpinellifolium* as an alien invasive species.

Chapter 3 is divided into three sections.

In Chapter 3a I discuss the genetics of the wild collected Galápagos tomatoes as collected by myself during the extensive fieldwork in 2000 and 2002. The following questions were addressed:

1) Can the four different species of tomato from the Galápagos Islands described using morphological characters, be discriminated using genetic markers and how does this correspond to the species circumscription outlined in Darwin *et al*. (2003)?
2) What genetic diversity is there within and between populations and taxa?
3) Is there any genetic evidence of hybridization between the taxa and is there congruence or incongruence between the genetic and morphological data?

Chapter 3b is an analysis of populations containing putative hybrids as established in the field in the Galápagos Islands. The following questions were addressed:

1) What genetic or morphological evidence is there to support the hypothesis of hybridization between tomato species in the Galápagos Islands?
2) Do results from genetic markers and morphological analysis give congruent results and provide evidence of hybridization between the different species of tomatoes growing in the Galápagos Islands?
3) How do the morphometric delimitations of *S. cheesmaniae* and *S. pimpinellifolium* and their hybrids from Baltra Road on Isla Santa Cruz correspond to the results from allozyme electrophoresis?
4) What recommendations do these results suggest for the future conservation of Galápagos tomatoes?

Chapter 3c is an analysis of the seed bank accessions from TGRC and Cornell University. The following questions were raised:

1) Can the four different species of Galápagos tomatoes from the two seed banks be discriminated using genetic markers and what is the diversity within the collections?
2) How does the genetic analysis of the seed bank accessions compare to the more recent wild collections made by me during 2000 and 2002 fieldwork?
3) Is there evidence of hybridization within the seed bank accessions?

Finally, in Chapter 4, I present the overall conclusions of the study. This brings the research into the wider context of historic and current understanding of endemic island species, invasive species, hybridization and conservation of wild crop relatives. I suggest possible applications of the present work, outline a future work direction and make conservation suggestions for the future preservation of the endemic Galápagos tomatoes.

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Chapter 1

Plant invasions on oceanic islands

History of invasive plants research

Humans have transported plants and animals around with them for millennia. Early human migrations and trade have led to the movement of many cereal crops and their associated pests (Mack *et al*., 2000). Invasive plants are now ubiquitous in all ecosystems and there is now no nature reserve without alien plants (except in Antarctica) (Lonsdale, 1999).

Alien invasive plants have been documented for over 150 years by, for example, De Candolle, 1855; Darwin, 1859; Hooker, 1864; Franchet, 1872; Goeze, 1882; (cited in Rejmánek *et al*., 2005a). However, the publication of Elton's (1958) book, *The Ecology of Invasions by Animals and Plants*, is widely recognised to have been the beginning of the research now known as invasion ecology (Richardson and Pyšek, 2006).

The SCOPE Programme (Scientific Committee on Problems of the Environment) biological invasions, and the subsequent publications including a book edited by Drake *et al*. (1989), marked the start of an explosive growth of research in invasion biology. This SCOPE programme aimed to raise research interest into invasive organisms and to encourage the top ecologists to direct research in this area. It had three fundamental questions – 1) what factors determine which organisms invade and which do not? 2) which habitats are most prone and which are most resistant to biological invasions? and 3) with this new found knowledge, how can invasions be managed (Drake *et al*., 1989)? In this same publication a paper by Rejmánek (1989) on invasibility of plant communities raised the questions of the importance of "disturbance, competitiveness, the re-colonisation ability of native species and the quantity of imported aliens' propagules in the whole invasion process". This paper was groundbreaking at the time (Krahulec *et al.,* 2006) and the same questions are still being asked 20 years later. Although progress has been made in this area

the questions are as interesting and as relevant as ever due to the increased level of habitat fragmentation and levels of plant introductions. The SCOPE publication did bring invasive species to the forefront but did not provide practical advice on how to deal with these organisms. A more recent SCOPE publication aimed to rectify this (Mooney *et al*., 2005).

Current research into the ecology of alien invasive plants can be categorised into three main areas – invasiveness, invasibility and the impacts of invasions (Alpert *et al.,* 2000). Lonsdale (1999) also writes that there are three different themes to invasion ecology 1) ecosystem properties (including ecosystem resistance and disturbance of the ecosystem; 2) propagule pressure and dispersal; and 3) properties of both the native and the exotic species. Applied research aims to eliminate/control the invasive individuals or their impact and the more theoretical research - to enable predictions of future problem plants – for example Rejmánek and Richardson (1996). Invasive organisms have also been studied to elucidate some basic processes in population biology (for example Meekins *et al.,* 2001).

Most plant introductions are deliberate rather than accidental. Mack and Erneberg (2002) estimated that the majority of naturalised plants in the United States were introduced intentionally. This is not the case for other groups of organisms; for example, with invertebrates the reverse is true (Tye, 2006). Most invasive plants were first grown in cultivation (either agricultural or horticultural) and from there they become naturalised.

Alien invasive organisms in general (including agricultural weeds) are now widely thought to be one of the most serious ecological and economic threats (Cox, 2004 and references therein). On top of the widely accepted economical threats, invasive species in general can cause considerable damage to natural ecosystems; they can, for example, drive large scale alterations of ecosystem function, cause extinction, or reduce populations of native species. Damage can be caused by different mechanisms; for example, predation, competition and hybridization (Williamson, 1996; Ludsin and Wolfe, 2001). The Millennium Ecosystem Assessment Biodiversity Synthesis Report (http://www.millenniumassessment.org/documents/document.356.aspx.pdf)

identifies invasive species as one of the five main drivers of change in biodiversity and ecosystems.

Alien vascular plant species comprise 5%-25% of the floras of many nature reserves in the United States and 50%-70% in Hawaiian nature reserves (Ludsin and Wolfe (2005).

The biggest threat to biodiversity on oceanic islands is now thought to be invasive species (Tye, 2006). For example the Convention on Biological Diversity (CBD) recognises the importance of addressing invasive species in isolated and vulnerable ecosystems (de Poorter, *et al*., 2005) and the Millennium Ecosystem Assessment Biodiversity Synthesis Report the impact of invasive species on islands is described as being in the highest impact category - "very high" and it is considered that it will continue to be very high in the future (see p. 16, Millennium Ecosystem Assessment, 2005 http://www.millenniumassessment.org/documents/document.356.aspx.pdf)

Tens rule sensu Williamson (1996)

The Ten-ten rule, as it was originally called, was put forward by Holdgate (1986); and Williamson and Brown in 1986 (Williamson 1996). Including the later additions, the "tens rule" (as it is now called) states that 10% of imported plants become feral ("introduced" *sensu* Williamson, 1996 see table 1.1), 10% of feral plants become established (naturalised) and 10% of established plants become pests (with a negative impact; see Table 1.1). The tens rule was originally based on data derived from alien plants in Britain but a similar rule holds true in many temperate and Mediterranean areas (Williamson, 1996). There are, however, many areas where this rule does not fit - for example on oceanic islands. In the Galápagos Islands the number of alien pests is thought to be much higher (M. Rejmánek *in litt,* 2003.).

Williamson (1996) alerts the reader to the fact that his word "established" (see below) refers to the establishment of a species in any habitat and not necessarily a natural habitat. He also raises his concerns that the terms for invasive species are source of uncertainty. For example he defines both "introduced" and "established" as being feral. However I feel feral refers more

closely refers to Williamson's "introduced" and naturalised refers to Williamson's "established".

Since the tens rule was originally published it has received much attention. Ludsin and Wolfe (2001) noted that the figure for invaders should be higher than 10% and more like 38%. However they note that this may be too high many studies do not (or cannot) include failed invaders in the calculations. Different areas and habitats deviate from this rule. The conditions that an introduced plant is exposed to vary considerably, so in some ways it would be surprising if there would be a fixed law on levels of survivorship in introduced and invasive plants species. However I believe the tens rule can still provide a useful framework to use as a basis of comparison between different places.

Table 1.1 Tens rule (*sensu* Williamson 1996). Each transition has a probability of around 10% (5%-20%) (Williamson 1996). Williamson's titles for each of the terms are in bold with his comments in parentheses

Williamson's terms	**Preferred terms used in this thesis**
Imported (brought into the country)	Introduced
"Introduced" (found in the wild but not breeding freely, A.K.A. casual or feral)	Casual
Established (self sustaining populations, breeding freely, A.K.A. naturalised and feral)	Naturalised
Pest (with negative economic impact)	Invasive or Transformer

Invasive species terminology

The variation of terms used by Williamson (1996) to describe invasive species raises the issue of how important it is to have consistent, usable and understandable invasive species terminology. Inconsistencies in invasive species terminology not only affect the estimates of invasive species but also make it confusing for the wide variety of people that are involved with invasive species and their consequences; these people include, for example, social and natural scientists, ecologists, conservationists, taxonomists, those involved with compiling floras, land managers and even politicians. Colautti (2005) stresses the need for consensus on terminology and uses the example of the term 'invasion success' that has been used throughout the literature in three different

contexts – 1) establishment of an invasive, 2) spread of an invasive species (increase in range), and 3) proliferation of an invasive species (increase in numbers).

Plant invasions are thought to take place in a continuous, rather than a stepped sequence; from casual to naturalisation to invasion and transformer (*sensu* Richardson *et al*., 2000 see Table 1.2). This makes finding the correct term to describe a particular (alien) plant at a particular time period a difficult and subjective task.

There are many different names given to alien species. Lockwood *et al.* (2007) included 27 different terms to describe alien species. Different, often overlapping, terms and words are scattered throughout the literature. To name but a few: alien pest species (Rejmánek and Randall, 2004); alien plants (Richardson *et al*., 2000); anthropogenic introductions (Emerson and Kolm, 2005); environmental weeds (Richardson and Pyšek, 2006); exotic species (Lonsdale, 1999); non-native edificators (Rejmánek *et al.,* 2002); invasive plants (Henderson *et al*., 2006); nonindigenous species (Colautti *et al*., 2006); Exotic (non-indigenous) species (Ludsin and Wolfe 2001); invasive alien species (CBD http://www.cbd.int/programmes/cross-cutting/alien/); harmful nonindigenous species (a report cited in Rejmánek *et al*., 2002);non-native invasive plant species (DeWalt *et al*., 2004); pests (Richardson and Pyšek 2006); weeds (Denslow, 2003).

The different terms used to describe alien plants and the apparent continuous nature of plant invasion makes it difficult to establish accurate and consistent numerical estimations of alien plants. This causes inconsistencies in the data and literature (Daehler, 2006) and can undermine the management of invasive species (Colautti and MacIsaac, 2004).

Efforts have been made, in recent years, to gain some clarity in terminology for invasive plant species; see for example, Rejmánek *et al*. (2002) and references therein; Williamson (1996); Richardson *et al*. (2000); Davis and Thompson (2000), Davis and Thompson (2001); Daehler, (2001b); Pyšek *et al*., (2004); Colautti and MacIsaac, (2004); Colautti, (2005).

Davis and Thompson (2000) suggested that there are eight types of plant colonisers. They identified two types that should be described as invaders. Short distance dispersers (Type 4 *sensu* Davis and Thompson, 2000) and long distance dispersers (Type 8 *sensu* Davis and Thompson, 2000) both novel to the area and having a large impact on the invaded region. Davis and Thompson (2001) put forward the argument that there were practical reasons to include impact. Their inclusion of the importance of the impact of alien plants as a criterion has been criticised by Daehler (2001b) and Pyšek *et al*. (2004) for making definitions too subjective. Daehler (2001b) writes that in ecology the term 'invasion' can be used to describe a native species arriving and spreading as part of natural succession and that invasive species terminology should be consistent with ecological terminology and not used as Davis and Thompson (2001) suggest referring to novel species with negative impacts. Rejmánek *et al*. (2002) also comment that the term 'invasion' should not necessarily have the negative connotation that Davis and Thompson (2001) infer. Chew and Laubichler (2003) warn against misconceptions because many terms used in ecology and invasion biology are metaphors.

It seems sensible at this stage to refer to both general and specialist dictionaries to establish the recognised definition of 'invasive'. The etymology of the word 'invasion' can be traced back to classical Latin (4c.) where the word came from invadere "to go into, fall upon, attack, invade" from *in*- "in" + *vadere* "go, walk" (http://www.etymonline.com/index.php?search=invade&searchmode=none).

In the Oxford English Dictionary (Simpson and Weiner, 1989) for example, all the general definitions of 'invasion' infer that the effect of an invasion is harmful in some way – for example "action of invading a country or territory by an enemy" and "spreading of pathogenic micro-organisms" and "A harmful incursion of any kind". However under the subtitle *Ecology* in the Oxford English Dictionary (Simpson and Weiner, 1989) the definition for 'invasion' is more neutral "the spread of a plant or animal population into an area formerly free of the species concerned". There are references below referring to the literature where the term 'invasion' in this context was first used. The first of

these refers to “1905 F. E. Clements. Res. Methods Ecol. iv. 210. By invasion is understood the movement of plants from an area of a certain character into one of a different character and their colonization in the latter”. Thus there is no mention of the alien nature of invasions or negative impact.

However definitions of ‘invasive’ in more specialist dictionaries/glossaries often mention negative impacts and refer to the invader as being non-native. A sample of examples is listed below:

- *Dictionary of Environment and Ecology* (Collin, 2004): “Invasive - Referring to an organism that enters an area in large numbers especially a non-native species that threatens ecosystems and habitats or other species”.

- *The MacMillan Dictionary of the Australian Environment* (Meagher, 1991): “Invasion – The spread and establishment of pest plants or animals into an area where they have not previously been known”.

- *The Oxford Dictionary of Environment and Conservation* (Park 2007) “Invasive species – An aggressive introduced species which spreads and dominates its new location, competing with and often replacing native species and proving difficult to remove”.

- “Invasive plant – a non-native plant species that is able to spread on its own causing environmental or economic harm” (http://aquat1.ifas.ufl.edu/glossary.html).

Some dictionaries/glossaries, however, have a more neutral tone; for example see examples below:

- Gurevitch *et al*. (2006) “invasive species – a species that is rapidly expanding outside of its native range”;

- Lincoln *et al*. (1998) “Invasion – the mass movement or encroachment of organisms from one area to another”;

- Owen (1962) “Invasion - the migration and establishment of an organism in a new location”.

Other dictionaries have no references at all for the term invasive for example: *The Oxford Dictionary of Ecology* (Allaby, 2005), *Henderson’s Dictionary of Biological Terms* (Holms,1979), *Dictionary of Environmental Science or Technology* (Porteons, 1996) and *McGraw-Hill Dictionary of Life Sciences* (Lapedes,1996) only includes a reference for medical terms under invasion. There is even a discrepancy in the terminology for alien invasive species in the International Union for the Conservation of Nature (IUCN) literature on line. The IUCN “Guidelines for the Prevention of Biodiversity Loss Caused by Alien Invasive Species” (http://www.iucn.org/themes/ssc/publications/policy/invasivesEng.htm#anchor392619) prepared by the Species Survival Commission (SSC) Invasive Species Specialist Group (ISSG) there is a section titled “Definition of Terms”, where “Alien Invasive Species” means “an alien species which becomes established in natural or semi-natural ecosystems or habitat, is an agent of change, and threatens native biological diversity”. However in the IUCN ISSG global invasive species database, invasive species are referred to invasive alien species with the words alien and invasive the other way round! As do the CBD (Convention of Biological Diversity). (http://www.issg.org/database/species/search.asp?st=100ss&fr=1&str=; http://www.cbd.int/)

Elton (1958), in his seminal work on invasive species never defined *invasion* or *invasive* although many (but not all) of his examples were for introduced organisms that had a negative impact on the invaded region. It appears as if ecologists in general do not agree on the terms for invasion let alone invasion biologists/ecologists. It is however quite clear that the non-specialist dictionary term ‘invasion’ infers some sort of negative impact. So it is not surprising that in the biological/ecological context many would assume that this term makes some statement of impact. In addition to this, the lack of inclusion in several dictionaries (including one specifically for ecology) would indicate that the term invasion may not be so widely used in ecology to describe an invasion as part of a natural succession as suggested by Daehler (2001b).

Richardson *et al*. (2000) have compiled a usable and thorough list of invasive species definitions (see Table 1.2). They propose that the term invasive should be used to describe "introduced plants [that] produce reproductive offspring in areas distant from sites of introduction". The term invasive, they write, should not imply an economic or environmental impact and that the words pest and weed are suitable for this. They suggest that the term transformer should be used to describe the 10% of invasive plants that change the "character, condition, form or nature of an ecosystem over substantial areas".

Richardson *et al*. (2000) raise concerns that the term naturalized is used to describe different situations in the literature. For example they found that 29% of the (157) papers they looked used the word naturalized as a synonym for invasive. They suggest a of combination of reasons - the historical use of the term naturalised (e.g., Charles Darwin) was used to describe introduced taxa behaving like a native; in addition to this usage, later confusion has been caused over the use of these ecological terms in the non-English speaking regions. Richardson *et al*. (2000) suggest that the term colonisation should replace the term invasion when referring to the succession of native vegetation. They consider most ecologists use the term 'invasion' for alien plants rather than natural succession of native species. It would be interesting to make an assessment of this within the general ecological literature.

Table 1.2 Terms to describe alien plants suggested by Richardson *et al*. (2000).

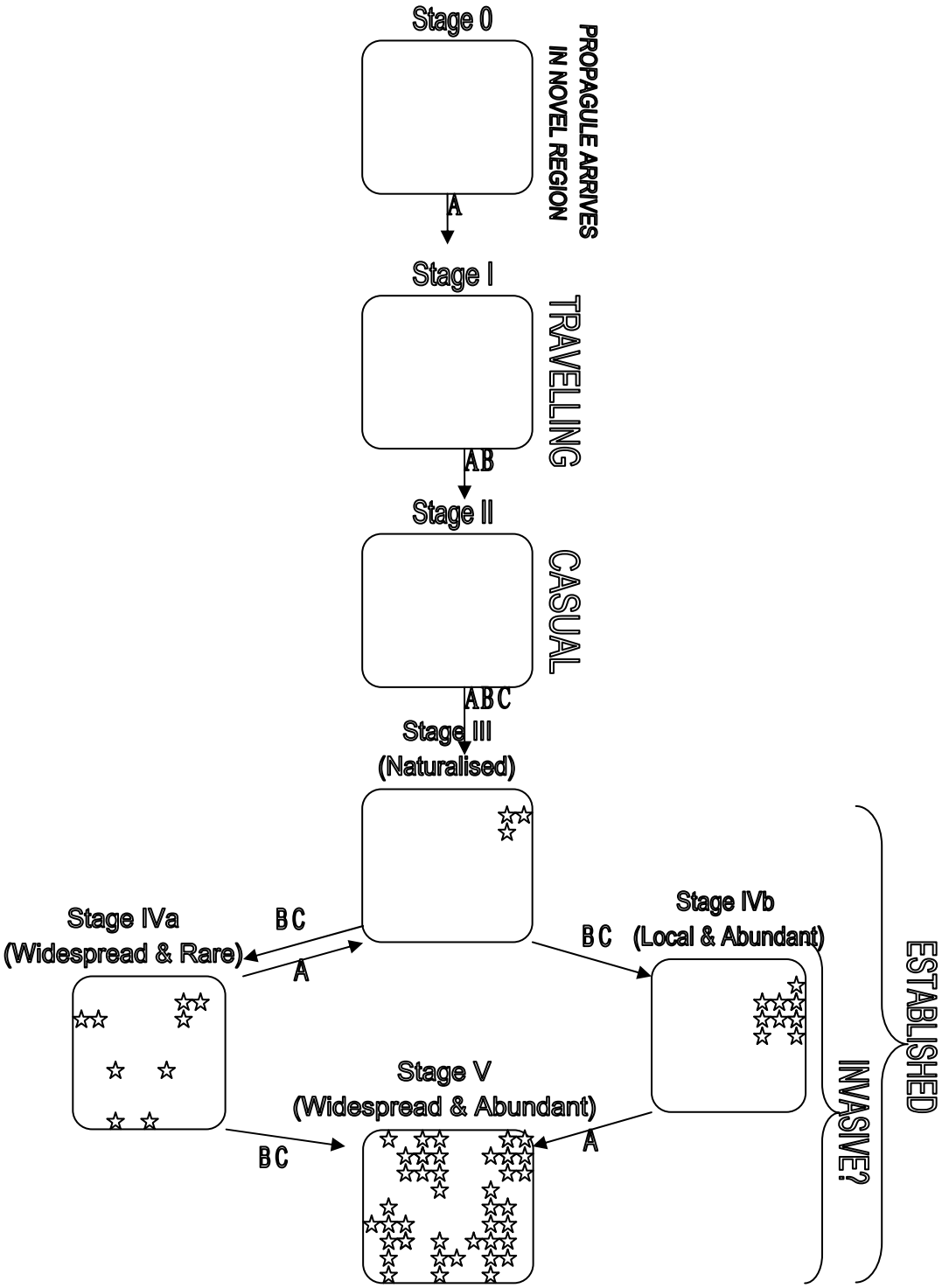
Category	**Description**
Native plants	A taxon that originated or arrived in an area without human involvement. (Excluding taxa that arise as a result of hybridization with an alien plant)
Alien plants	Plants whose presence is due to intentional or accidental introduction as a result of human activity.
Casual alien	Plants that may even reproduce occasionally in an area but need repeated introductions to maintain populations.
Naturalised plants	Alien plants that consistently reproduce to form self perpetuating colonies, usually not far from the parent plant, this is not necessarily natural, semi-natural or human made habitats.
Invasive plants	Plants that regenerate naturally and produce reproductive offspring more than 100m from the parent plant in under 50 years in natural habitats for plants reproducing by seeds or other propagules (>6m/3years for plants reproducing vegetatively) with no reference to environmental or economic impact.
Weeds	Plants that are not necessarily aliens, but are growing in areas where humans do not want them. They usually have an economic or environmental effect.
Environmental weeds	Plants that invade natural habitats and usually have a negative effect on native biodiversity and or ecosystem functioning.
Transformers	Plants that change the “character, condition, form or nature of an ecosystem”

The definitions by Richardson *et al*. (2000) used in Table 1.2 can be criticised: 1) It may not be practicable to use this definition of ‘invasive species’, particularly as Richardson *et al*. (2000) write that >100m/ <50 y should not be interpreted as >2m/y. The date of establishment of the plant may not be known due to inadequate records. 2) The term weed has traditionally referred to a plant that causes an agricultural or horticultural problem thus ‘environmental weed’ could be confusing.

Colautti and MacIsaac (2004) go one stage further and propose a model framework for defining the invasion biology terms see Figure 1.1. Colautti and MacIsaac (2004) consider that a plant could be described as invasive in stages IVa and V and not stages III and IVb although some ecologists, Colautti (*in litt*., 2008) admits may consider a plant to be an invasive in stages IVa, IVb and V depending, of course, on their definition of invasive. The Colautti and MacIsaac (2004) (C&M) model was put forward to avoid exactly this type of confusion to enable invasion ecologists to be free of terms like invasive. I like the way that the C&M model recognises the continuous nature of invasive species. However this model too is without reference to impact.

An extremely useful addition by Colautti (2005) to the C&M model is that the different stages can be used to describe the same alien plant within the same area but in different habitats. The example that Colautti (2005) gives: road side – the plant might be dominant (either localised stage IVb or wide spread stage V), old field habitats – interspersed (stage III or IVa) and in forest under story - self-sustaining (stage III) (Colautti 2005). In Figure I have used the framework outlined in the C&M model and added graphic illustrations and descriptions to these different stages. This model could be very useful although I wonder how willing interested parties will be to change entrenched methods. The C&M model warns against using adjectives to define these types however I feel that using the description (e.g., widespread but rare) is easier than using the stage number (e.g., stage IVa) which readers would constantly need to refer to a reference or diagram interpret what is meant. I believe, in general, systems used to describe invasive species are very important for several reasons 1) to allow effective communication between interested parties, 2) it reduces some of the subjectivity in descriptions of invasives and 3) systems can help to set conservation priorities.

Figure 1.1 Transitions of an alien species from the point of introduction into a novel region to a widely distributed and dominant alien invasive species (adapted from Colautti and MacIsaac, 2004).



Additional explanation of Fig. 1.1

The filters are represented by the letters **A**, **B** and **C** placed by each arrow. These indicate the filter factor that determines whether the potential invader will pass through to a subsequent stage. **A**- propagule pressure, **B**-physicochemical requirements of the would be invader (e.g. resource availability); **C**- community interactions (Colautti and MacIsaac, 2004).

The Stages start at Stage 0 representing the introduction of a propagule into a novel region. The boxes at Stages III – IV contain stars that represent hypothetical established populations at different levels of distribution and density. Stage III – local and rare, Stage IVa – widespread and rare, Stage IVb – local and abundant and Stage V- Widespread and abundant. Colautti and MacIsaac (2004) stressed that adjectives should not be used in lieu of the Stage number and they purposely avoided using terms like established in their version however for clarity I felt that this was necessary. In my opinion it would be easier to refer to a stage as local and abundant rather than Stage IVb.

Note that the filters B and C may positively or negatively affect the number of propagules that are able to pass through. For example the C filter (community interactions) might represent the presence of a beneficial pollinator or mychorrizal fungi or conversely a predator causing a negative effect (Colautti and MacIsaac, 2004). This diagram does not include levels of impact of the invasive species.

Rejmánek *et al.* (2005b) also suggest that invasive plant species that are having a negative impact should be distinguished by being called something different. They suggest either the term exotic weeds or exotic pest plants.

As already mentioned Daehler (2001b) have suggested that nearly all invasive plants will have some impact, but I believe that the scale of the impact needs to be established. Several authors have written that invasive species should not be defined on their impact. Establishing the impact of invasive species, however, is essential for economic reasons and to prioritise conservation efforts. Using the C&M model there could be examples where a stage IVb plant is having a large impact locally but according to some definitions should not be described as invasive. The impacts of invasive species are relative and in addition to this can be viewed differently by different groups of people with different interests. For example a hydrologist might decide that an invasive plant species that changes the hydrology of an area is a serious impact; whereas a forester might be more concerned by an invasive tree species. In reality impacts of invasive species are often much more complex and involve multiple effects. In areas with high levels of invasive species, priorities have to be established for

practical and economic reasons. I think it might be useful to have a scoring system to help calculate the current impact and estimate the potential impact of an invasive plant species more objectively. This could then be weighed up against the feasibility of eradication/control of the invasive species and potential cost thereof. In Table 1.3 I proposed a new scoring system for impact/threat for invasive plant species.

I think that it would be useful if an alien plant species in a particular area could be described using a maximum of three different criteria. In Table 1.4 I assess the utility of my new threat/impact system by scoring *S. pimpinellifolium* as an invasive species in the Galápagos Islands.

In the ten Habitat categories (Table 1.3) *S. pimpinellifolium* (from Baltra Road on Isla Santa Cruz) scored a total of 49/50: 1) susceptibility score– 5; 2) habitat score– 4; 3) areas of conservation concern score– 6; 4) ≥25% endemic species score – 6; 5) in area of rare/endangered species score– 5; 6) occurs in more than one area scores – 5; 7) occurs in more than one habitat scores– 5; 8) proximity to natural habitat scores – 4; 9) >100m from source scores – 5; 10) in area with anthropogenic disturbance score - 5.

In the five invasive species categories *S. pimpinellifolium* scores a total of 13/25 : 1) native range similar score– 5; 2) known to invade other areas scores – 0; 3) propagule pressure (unknown) score – 0; 4) years since introduction >10years scores –3; 5) in region of congeneric score - 5). Combining both the habitat and the invasive species categories gives a total of 63/75, thus it poses a high threat.

Table 1.3 Proposed outline for invasive plant species threat/impact scoring system. I propose two scoring categories: 1) habitat and 2) invasive plant species. The total from each should then be added together to make a 'threat/impact score'. There could also be a similar system to score for impacts on environmental services. * 100 of the worlds worst invasive alien species (IUCN) (http://www.issg.org/database/species/search.asp?st=100ss). Bold scores indicate those that contribute to the maximum potential

Habitat	**Score**	**Invasive plant species**	**Score**
1) Region known to be susceptible to invasive species (e.g. island)	**5**	1) Invasive plant's native range similar to invaded range in habitat/climatic type	**5**
2) Habitat known to be susceptible to invasive species (e.g. mesic environment)	**4**		
3a) Region is an area of particular importance (e.g. National Park, RAMSAR site, World Heritage Site, SSSI etc.)	**6**	2a) Invasive plant species known on invasive species register (* 100 of the worlds worst invasive alien species IUCN)	**5**
OR		OR	
3b) Region is within close proximity to an area of particular national/international importance e.g. National Park, RAMSAR site, World Heritage Site, SSSI etc.	4	2b) Invasive plant species known the be invasive elsewhere in the world in a similar habitat but not in top invasive species register (* 100 of the worlds worst invasive alien species IUCN)	4
4a) Invaded habitat with ≥25% endemic species	**6**	3) Propagule pressure high i.e. near a source of propagules or invasive species being introduced regularly	**5**
OR			
4b) Invaded habitat near habitat with ≥25% endemic species	5	4a) Years since introduction approx >25 years OR	**5**
5a) Invaded habitat with rare/endangered species	**5**	4b) Years since introduction approx > 10 years	3
OR			
5b) Invaded habitat near habitat with rare/endangered species	4	5) Invasive plant species in area with congeneric native species raising the potential of hybridization	**5**
6) Invasive found in more than one distinct area	**5**		
7) Invasive found in more than one different habitat type	5		
8a) Invasive found in natural habitat	**5**		
OR			
8b) Invasive found near natural habitat	4		
9) Invasive plant > 100m from source	**5**		
10) Area with anthropogenic disturbance	**5**		
Total potential	**50**	**Total potential**	**25**

Table 1.4. The alien plant definition, stage and 'threat/impact score' for *Solanum pimpinellifolium* in the Galápagos Islands

Plant Species, Habitat and Locality	**Alien Plant Definition** (*sensu* Richardson *et al.,* 2000)	**Stage (C&M model)** (*sensu* Colautti and MacIsaac, 2004)	**Threat/impact score** (see Table 1.3 above for definitions)
Solanum pimpinellifolium **Open scrub land, roadside, forest margins Galápagos Islands**	'Invasive'	'V' (i.e., widespread and abundant)	62/75 (i.e., high threat/impact)

In conclusion, I find the term 'harmful nonindigenous species' unnecessarily long winded. I believe the addition of the word alien to the term invasive (either alien invasive species or invasive alien species) is unnecessary as, by virtue of the fact an invasive *is* an alien (i.e., it is an outsider) according to many, if not most, dictionary definitions. Personally, I prefer the term invasive to describe an alien plant that is either causing a problem or has the potential to cause a problem in a natural/semi natural habitat again because an invader according to the dictionary does have a negative impact. For the purposes of this thesis I use definitions *sensu* Richardson *et al*. (2000). This is with the caveat that I merge their terms "environmental weed" with "invasive" and use the term 'invasive'. In addition to these, the terms for the stages (C&M model) outlined by Colautti and MacIsaac (2004) can help to build up a useful picture of the density and distribution of specific populations of invasive plants.

Are some areas more susceptible to invasive species than others?

Charles Darwin (1859) observed that habitats varied in their numbers of naturalised species. There has been much research to establish if some regions are more susceptible to plant invasions than others and if so which habitats/regions are most vulnerable.

Assessing invasive species is complicated, as already discussed. The same is true when assessing the invasibility of an area. It is important to discriminate between invasibility (i.e., the vulnerability of an area to invasion) and the level of invasion (i.e., the number of invasive species in an area) (Richardson and Pyšek, 2006). For example, variations in the latter might be as a result of more species having been introduced in the first place.

In general it is thought that mesic environments are the most vulnerable to invasions. More xeric habitats are not conducive to seedling germination and survival and in the wet terrestrial habitats there is intense competition with other plants (Rejmánek *et al.,* 2005a).

To know if a particular area is intrinsically more invasible, levels of immigration and extinction (of the introduced species) need to be established. *E=IS,* where *E* is number of exotic species, *I* number of introduced exotic species and *S* the survival of the exotic species in its introduced new range. In reality accurate accurate estimates of *I* and *S* are often unknown (Lonsdale, 1999). Lonsdale (1999) suggests that *I* should be broken down into accidental and intentional introductions and that *S* should be broken down into four different categories of exotic species survival after extinctions due to: 1) competition with native vegetation, 2) herbivory and pathogens, 3) chance events (e.g., drought) and 4) maladaptation (e.g., a temperate plant introduced into an area with a tropical climate). This equation does not take into consideration area size; larger areas carry more exotic species. He suggests that *E* and *I* are therefore scale dependant. Numerous studies (see Lonsdale, 1999) have used percentage exotics instead of area (i.e., number of exotics/number of natives), although there are concerns over this (Lonsdale, 1999).

Lonsdale (1999) used this method to compare regions to establish if some areas were more easily invaded than others. The results showed that: 1) exotic species richness was positively correlated with native species richness; 2) New World regions were more invaded than Old World (but only when native species richness is factored in); 3) mainland temperate sites were more easily invaded than mainland tropical sites but this is not the case with temperate and tropical islands; 4) islands have higher levels of invasive species but this is not explained by the fact that islands are less diverse than mainland counterparts; and 5) areas with higher levels of human visitors increases numbers of invasive species. However Lonsdale (1999) proposes that this last effect is due to increased disturbance rather than increased propagule pressure.

Are islands more susceptible to invasive plant species than other areas?

Islands are thought to be particularly susceptible to invasive species (Herben, 2005 but see Sol, 2000). Lonsdale's study (Lonsdale 1999) found that islands had nearly three times the level of "exotic" plants as mainland sites. Many theories have been put forward to explain why islands appear to be so susceptible to plant invasions. Denslow (2003) divided the invasibility of an area into three sections: 1) opportunity; 2) invasibility; and 3) impact.

Opportunity

Are there simply more plants introduced into islands in the first place and therefore more opportunities for invasions to take place?

Native island floras often lack essential food, fibre and fuel crops (Denslow, 2003) and plants that fulfil these requirements (along with their associated weeds and pests) are imported by settlers. Guézou *et al*. (2007) report that plants for medicinal purposes, shade trees and fence post are also often introduced to islands; for example in the Galápagos Islands the tree *Cinchona* sp. was introduced for quinine production. These are in addition to the

introduced farm animals along with their food crops, for example pasture and weeds therein.

Due to the isolation of some islands, settlers may take additional plants with them to remind them of 'home'. In Australia and New Zealand Plant Acclimatisation Societies were formed in the nineteenth century. These were primarily started to help settlers find appropriate crop species that would suit their new homelands but possibly also to make their surroundings more familiar. Landowners were encouraged to introduce plant species, on their recently acquired land in Australia, for "proof of possession" (M. Bourke pers. comm., 2006)

With increased expertise in artificial plant selection and a wider knowledge and understanding of plants, it is likely that humans are today more proficient at knowing more precisely which plants will suit which habitats and therefore perhaps not surprising that more recently a higher proportion of introduced plants have become naturalized.

Islands in general also probably have higher rates of accidental plant introductions from passing ships using islands as drop off points (Denslow, 2003). This is unlike the equivalent isolated mountaintop 'island' for example.

Invasibility

Are islands intrinsically more susceptible to invasions by plants? Below I briefly introduce several theories as to why islands and indeed other isolated areas may be more invaded than others areas.

Species richness

Elton (1958) used islands and their vulnerability to invasive organisms to support his theory that species-poor areas are particularly vulnerable to invasions. Elton's theory became ecological dogma. It was proposed that species diversity led to ecosystem stability (see Levine, 2000). Elton's theory raises three relevant questions: 1) are species poor areas more vulnerable to invasions? 2) are islands more species poor?; and 3) if islands are more

species poor relative to other areas, does this effect their vulnerability to invasions?

1) Are species-poor areas more vulnerable to invasions?

Elton's theory of the vulnerability of species-poor regions to invasions has been supported by experimental research (manipulation of species numbers in experimental plots) (Denslow, 2003). Levine (2000) questioned the relevance of this type of research to natural communities. He undertook some experimental research, manipulating species diversity *in situ* in the Californian riparian zone. His results, however, supported the theories that the more diverse the habitat the more invasible it is. Several observational studies have found correlations between native species diversity and non native species diversity on islands (see Lonsdale, 1999; Williams and West, 2000; Daehler, 2006). Denslow (2003) gives numerous examples of other studies that find similar results for other non-island habitats.

Several authors (Kühn and Klotz, 2007 and references therein) have suggested that the relationship between native plant species richness and alien plant species richness is area dependant. To test this theory they compared three different plots sizes. The smallest plot was a 'point scale' (number of native and alien plants that regularly touched a fixed stake). Here they found that there was a negative relationship between native and alien species richness (i.e., the more native species the less alien species). In $20m^2$ plots they found no relationship between native and alien species richness. In the larger plots - $30km^2$ they found that there was a positive relationship between native species richness and alien species richness (i.e., the higher the native species richness the higher the alien species richness). They suggested that the positive relationship between native and alien species was caused by 'common large scale environmental factors driving species richness (Kühn and Klotz, 2007). It is important to note that Kühn and Klotz (2007) were looking at the whole spectrum of alien species and not only invasive species.

Conclusions have been drawn that the same factors that make areas rich in natives also make them capable of being rich in non-native plants (Denslow, 2003). In addition to this, some studies have shown that there is no relationship

between species richness and the invasibility of a community (see Rejmánek *et al*., 2005a).

Fridley *et al*. (2007) refer to these contradictions, for example between experimental and observational research results, as “the invasion paradox”. They state that there is a positive relationship between native species and “exotic” diversity at the broad scale whereas there is a negative relationship at the fine scale (similar to Kühn and Klotz, 2007). They conclude that native-rich ecosystems are likely to be rich in “exotic” species and that a reduction of species richness can increase the rate of invasion. Denslow (2003) concludes that these differences between observation and experimental results should be “resolved considering process and scale”.

2) Are islands more species poor?

A few years after the publication of On the Origin of Species (Darwin 1859), Joseph Hooker presented a paper at the British Association on insular floras. Here he described island floras as having the following characteristics – endemicity, impoverishment, dispersal and disharmony (Berry, 1998). In 1967 MacArthur and Wilson expanded their 1963 island equilibrium theory into a book entitled *The Theory of Island Biogeography.* They proposed that the remoteness and the size of an island would determine the number of species that an island could carry. An island species’ immigration and extinction rates would reach equilibrium (MacArthur and Wilson, 2001). Smaller, more remote, islands would have fewer species than larger islands close to continental land masses. The reason for this, they speculated, was due to the fact that smaller islands would be capable of holding smaller plant population sizes and remote islands would be subjected to low levels of immigration (Denslow, 2003).

This book continues to be relevant today despite more recent detailed and sophisticated studies (MacArthur and Wilson, 2001). However there are many other factors besides size and remoteness that affect the number of species that occur on islands – the age, latitude, altitude, habitat diversity and productivity of the island as well as “patterns of species coexistence” (Denslow, 2003).

Although the MacArthur and Wilson island biogeography theory has become widely accepted, several studies using species/area graphs have shown that islands are not more species-poor than mainland areas of a comparable sizes (Denslow, 2003; but see Herben, 2005).

Some tropical islands have large numbers of endemic plant species, often within small geographical ranges and often con-generic species ranges do not overlap. This makes the within-stand species number lower (the level at which plants interact) on islands. Conversely, mainland tropical areas have high diversity within stands and congeneric species often overlap. This means that at a stand level species richness is likely to be lower on islands than the mainland (Carlquist, 1974; Denslow, 2003). Loss of dispersibility in island plants as discussed by Carlquist (1974) is common. This may further prevent allopatric congeneric species from interacting.

Denslow (2003) uses the example of 53 species of *Cyrtandra* (Gesneriaceae) from Hawai'i to illustrate the relationships outlined above. These species often have very restricted and non-overlapping ranges, which can make the stand level of species diversity lower while the archipelago diversity appears richer.

In addition to this taxonomists may have a tendency to overestimate the number of endemic congeneric species growing on islands due to their obvious morphological differences. The use of a morphological species concept on islands may have made islands appear artificially species rich. Due to geographical isolation island congeneric species may not have the opportunity to interbreed but can be genetically similar due to recent diversification. The wider availability of genetic testing may redefine some morphological distinct species as subspecies or forms of the same. Thus in reality islands may be even more species poor than they appear.

When trying to establish if species richness affects the invasibility of an area it may be helpful to look at functional groups instead of species richness (see below discussion regarding vacant niche theories).

Early human settlements on islands have also obscured original plant communities and species diversity due to prehistoric extinctions, habitat alteration and introductions (Denslow, 2003). This makes it difficult, in many instances, to determine the constituents of the original plant communities. Research using pollen core analysis can help to reconstruct past plant communities. This however is only possible when the conditions are suitable for pollen preservation (for example freshwater lakes or bogs). Kathy Willis from Oxford University and colleagues have taken core samples from Galápagos Islands from *Sphagnum* bogs, volcanic crater lakes and coastal lagoons. They will analyse the changes in vegetation over approximately the past 5,000 years with particular interest in the past 1,000, the period in which humans may have changed the landscape on the islands most profoundly (http://palaeoworks.anu.edu.au/Galapagos_report05.pdf).

3) Does the species richness of an island affect the island's susceptibility to invasions?

Most authors do agree that islands are more invaded than continental regions (see Londsale, 1999, Denslow 2003, Herben, 2005; but see Sol, 2000). The number of recorded extinctions thought to be caused by invasive species is higher on islands than in mainland areas (Simberloff, 1995). However there is much conflicting evidence in the literature as to whether species-poor areas are more invadable by invasive species than species-rich areas. Also there are conflicting reports as to whether islands are indeed more species-poor than mainland areas.

It may well be that it is not the relative species richness of an area that effects its susceptibility to alien invasive species but that there are other factors that are more important. Areas with high levels of environmental stress and extreme conditions can suffer from less invasive species (Kühn and Klotz, 2007). Some communities within islands seem not to be easily invaded by plants; for example the upland native forests of Hawai'i (Simberloff, 1995) and recent lava flows of Hawai'i (Denslow, 2003). I have not observed alien plants on new lava flows in the Galápagos Islands for example. However it seems likely to me that some of this apparent resistance to invasions might simply be lack of opportunity. Perhaps plants more suited to these more extreme conditions have never been

introduced. Indeed if one were to introduce a lava flow pioneer plant species (from a similar climatic region) to the Galápagos Islands, for example, one might find that it was more successful.

Species pool size *sensu* Herben (2005)

Herben (2005) suggests that it is not the species richness that differentiates islands from continental regions but the size of species pools. Species pools here are given to mean a "set of species whose propagules can reach the community in reasonable time and therefore have provided the species that occur in the given habitat". A larger species pool increases the chances that there will be a species that might be capable of excluding an invasive plant species. Islands by the virtue of their size have a smaller species pool and this might be a reason why small isolated islands are more susceptible to invasions than are continental regions.

Disharmonic flora (*sensu* Carlquist, 1974) and vacant niche theory (*sensu* Simberloff, 1995)

Carlquist (1974) described islands as having a disharmonic flora. He was not the first to describe this as Joseph Hooker, over a century earlier, had also described island floras as being in disharmony (Berry, 1998). Islands often lack functional groups, taxonomic groups and dispersal types (Denslow, 2003). Simberloff (1995) suggests that as a result of having lower species diversity on islands, there are entire gaps in "ways of making a living" in island biota. It is this disharmonic flora that causes vacant niches that the invasive species are thought to be able to exploit (Denslow, 2003). Simberloff (1995) explains that it is not the fact that islands are more species-poor than mainland areas (this, as already mentioned, is under dispute) that makes them more vulnerable to invasions. He suggests that this vulnerability is more likely due to the lack of entire groups of organisms on islands (for example, terrestrial mammals are often missing from islands). This absence can often mean that specific introduced species occupying a vacant niche survive and can have a large ecological impact. There is not an inherent lack of "biological resistance" to invasions that make islands more vulnerable to invasions. According to Denslow (2003) it is the low productivity of the disharmonic and depauperate floras that is to be blamed for island vulnerability of alien invasive species.

The demonstration of vacant niches has proved difficult (Mack *et al.,* 2000). Mack *et al.* (2000) also suggest that because immigrant plants will potentially find a lack of suitable symbionts and pollinators in their new found habitat this might have the reverse effect of providing protection for the islands. Rejmánek (in litt., 2003) suggests that the combination of depauperate floras and lack of mammalian herbivores on Islands leads to high vulnerability to invasions.

Shea and Chesson's (2002) community ecology approach came up with the 'niche opportunity' theory. The niche opportunity defines the conditions which promote invasions and is a combination of the effects of resource availability, natural enemies and the physical environment all of which vary in time and space. Niche opportunities vary between communities and are increased by disruptions. This theory is based on up-to-date niche theories (Shea and Chesson, 2002 and references therein).

Native species are poor competitors (sensu Denslow, 2003)

Simberloff (1995) considers that it is not helpful to make generic statements about the relative competitiveness of species on islands vs. those on mainland areas and that one should always be more specific about areas. He also comments that there is not necessarily a correlation between complexity and stability and in fact some simple systems are stable and vice-versa. However, island natives are usually described as being poor competitors. Several reasons have been put forward to explain this. Loss of dispersal mechanism and small seed banks could reduce the competitiveness of native plants. Another characteristic of oceanic islands is that their native plants often have wide ecological ranges (Denslow, 2003) and could be described as jacks of all trades and masters of none. This is in contrast with the theory that many of the oceanic island congeneric species have small ranges, as discussed above.

Resource use and resource-enemy release hypothesis (*sensu* Blumenthal, 2005)

Funk and Vitousek (2007) conducted a study of resource use efficiency (RUE) in invasive vs. native plants in Hawai'i. This was undertaken on plants found in three low resource habitats (i.e., habitats in which plant growth is limited by

water, light or soil nutrients). They found that the invasive plants were more efficient at using limited resources than the native plants in the short term and were “similarly efficient when RUE measures were integrated over leaf life spans”. These findings are contrary to the prevailing theory that native plants will out-compete alien plants in areas of low resources and that invasive plants only thrive in areas of disturbance (see below).

On islands low species diversity at the stand level is thought to lead to low resource use and thus resources are available for the establishment of new individuals (Denslow, 2003).

The resource-enemy release hypothesis states that alien plants that benefit from high resource environments, (i.e., require high levels of resources) are those that benefit from escaping their pest enemies (diseases and herbivores) in their native range (Blumenthal, 2005). Fast growing/ high seed producing plants are good colonisers. This theory states that high resource requiring species produce tissue at low metabolic cost and do not put resources into protecting their tissue from pests (Blumenthal, 2005). Reinhart (2006) comments however, that Blumenthal (2005) does not discriminate between specialist and generalist pest enemies. The use of biological control on invasive plants would greatly benefit from this type of research.

The fluctuating resource hypothesis proposes that aliens successfully enter habitats facilitated by low resource up take by native plants or high resource availability due to disturbance (also known as resource pulses, see Denslow, 2003). As far as the latter is concerned it remains to be seen if these plants continue to thrive in the absence of the disturbance (Funk and Vitousek, 2007). Poor dispersal rates often characteristic of islands might leave sites and resources available for invasive species (Denslow, 2003).

Enemy Release Hypothesis (Keane and Crawley, 2002)

The enemy release hypothesis (ERH) suggests that invasive plant species benefit from escaping from specialist enemies. This can act as a mechanism for its invasiveness. It also predicts that the generalist enemies found in the novel range of the invasive plant species will have a greater impact on the

native plant species giving added advantage to the invasive species. However some invasive species and their enemies can be transported together, for example seed-borne pathogens (Keane and Crawley, 2002). Islands might be even less likely to have enemies that will predate on invasive plant species due to the isolation and high levels of endemism found on islands.

Disturbance

The establishment of alien invasive species can be aided by both anthropogenic and natural habitat disturbance (Mack *et al*., 2000; Jenkins and Pimm, 2003; Colautti *et al*., 2006), especially the weedy species (Jenkins and Pimm, 2003). The success of invasive species in areas of disturbance is due in part to reduced levels of competition and increased availability of resources (Denslow, 2003).

Jenkins and Pimm (2003) found that many of the most serious invasive plants invaded disturbed habitats and also that many of the world's most disturbed habitats were host to high numbers of invasive species.

It has been suggested, however, that the reputation of alien invasive species as thriving in disturbed areas may, in part, be due to historical pathways of their introduction coinciding with the disturbance of habitats for agriculture more than a characteristic of invasive plant species (Denslow, 2003). The loss of dispersal ability of many island plants as described by Carlquist (1974) could further prevent native plants from taking immediate advantage of disturbance.

Urbanisation and agricultural disturbance have similar effects on the increase in richness of alien species. There are many papers (see Lockwood *et al.,* 2007 and references there in) showing the correlation between human population increase and alien species richness. However, Tye (2006) warns that until recently, alien plants were not routinely included in plant checklists/inventories and this rate of increase may be an artefact that gives the alien species accumulation graph an artificially steep recent curve.

Island endemic floras can be vulnerable to invasive grazing mammals; many mainland floras have coevolved in the presence of wild and domesticated

grazing mammals. Grazing animals are often introduced to islands (for example, goats in New Zealand and the Galápagos Islands) and their activities and disturbance can promote invasive plants and grazing tolerant herbs (Brockie *et al*., 1988). In addition to this, the habitat disturbance and in some cases thoroughly degraded landscape caused by introduced grazing animals can also be exploited by invasive plant species.

So, does the native vegetation re-colonise areas after the removal of alien grazing mammals? It might be that if/when alien herbivores are removed from an area, (as has been undertaken on several islands in the Galápagos where goats have been completely eradicated) there could be an opportunity for some invasive plant species to become more dominant. However this has not been documented in the Galápagos (F. Cruz and R. Atkinson pers. comm., 2008). I think that this could be an extremely interesting area in which to do some research.

Gimeno *et al*. (2006) conducted a comparative study of abundance and distribution of *Oxalis pes-caprae* (Oxalidaceae) between two Balearic Islands and two areas in mainland Spain. *Oxalis pes-caprae* is a geophyte native to South Africa and now found as an invasive in many Mediterranean and temperate areas. Their results showed that the occurrence of *O. pes-caprae* was consistently higher on the two islands compared to the mainland sites; however there was no difference in local abundance between islands and mainland sites. They suggest that their findings show that the biotic resistance of the native plant communities was less important than other “coarse-scale” aspects, for example human-mediated dispersal and the dependence of domestic animals. They also conclude that the fact that islands by their virtue cover a smaller area than mainland areas means that over a comparable time frame colonisation can take place in a wider variety of habitat types quicker on islands (Gimeno *et al*., 2006).

Historic/prehistoric exposure of plants to other biota (*sensu* Rejmánek *et al*., 2005a)

The isolation vs. historic/prehistoric exposure to non-native biota has been suggested as a reason why some habitats are more easily invaded and others

less so. In particular the isolation of some islands might make them more vulnerable to alien invasive plants as compared to large continental areas such as Eurasia. However this is, at present, little understood (Rejmánek *et al*., 2005a). It has been suggested that regions that have shared a long history with humans and their land use will have species that are pre-adapted to invasions (Kühn and Klotz, 2007). The islands most strongly affected by invasions are often found to be those that were least disturbed prior to colonisation by humans (Kühn and Klotz, 2007).

Darwin's naturalisation hypothesis

Charles Darwin (1859) wrote that there is more likely to be intense competition between congeners and thus naturalised plant taxa were more likely to belong to novel genera. Darwin clearly attributes this hypothesis to De Candolle however the theory is now known as Darwin's naturalisation hypothesis (DNH). Darwin (1859) used Asa Gray's flora of the NE United States (Gray 1848) as an example and stated that out of 162 naturalised plant genera "no less than 100" are non-native.

There has been renewed interest in DNH. Various studies have found evidence to support it (Richardson and Pyšek, 2006 and references therein) while others have refuted it. For example Daehler (2001a) studied the Hawaiian flora and his analysis of the native and naturalised alien flora showed that there were in fact more naturalised species that belonged to native genera than belonged to novel genera. Daehler (2001a) speculated that previous research that supported DNH was either anomalous or that the naturalised plant assemblage of Hawai'i and islands in general might depend on other factors as well. Others have since found similar results that refute DNH for example the New Zealand Flora (Richardson and Pyšek, 2006).

Short migrations by humans will result in more congeneric alien species. This is presuming that the humans would show a preference for introducing plants from their home country (as has been found in regions like Australia). Therefore the native range of the human settlers should be factored into this equation. As we have discussed, most plant introductions are not accidental and therefore the plants that are introduced to an area are not a random selection and introduce

biases to any analysis (Colautti *et al*., 2006). In addition to this, there could also be differences in the species introduced in modern eras compared to introductions made in former times.

In the Galápagos for example there are several naturalised/invasive congeners for example *Solanum* (Solanaceae), *Gossypium* (Malvaceae), *Psidium* (Myrtaceae) to name but a few (all native to mainland South America). It is possible, particularly for the latter two that these introductions were made to the islands because the settlers observed the successful presence of plants belonging to these genera as natives and made selections on this basis. It would be interesting to test the Galápagos Islands for DNH and apply similar statistical tests as undertaken by Daehler (2001).

Impact

It is also possible that the impact of invasive species on islands is more noticeable due the smaller scale of islands and that due to their high levels of endemism islands can often be in the public more spotlight than mainland areas.

Some islands, for example the Galápagos Archipelago, have only been settled relatively recently. Thus the deliberate plant introductions are also recent. It appears as if this archipelago is more invaded than other islands groups (M. Rejmánek pers. comm., 2002). It may indeed be that we are currently observing invasion events in the Galápagos Islands that in other areas took place hundreds or thousands of years ago.

It is thought that habitat disturbance aids the establishment of alien plant species (Denslow, 2003). Recent development and improvement of transportation to islands (e.g., aeroplanes and container ships) have made it possible to import equipment which can increase the scale and speed of disturbance in more remote islands and areas on islands. For example larger agricultural equipment can now be imported and road building equipment which means that homes can be built in more remote areas than was previously possible. This might increase the opportunities of invasive species to take advantage of the disturbance.

Propagule pressure

The term propagule pressure is used to describe the quantity of introduction events and the number of propagules per introduction event to a novel region, i.e., increasing the propagule number plus the number of introduction events increases the pressure. Propagule pressure is also sometimes called introduction effort.

The number of imported propagules of aliens has been discussed as an important aspect of the invasibility of plant communities (e.g., Rejmánek, 1989). The theory of propagule pressure aims to explain why some alien populations (not species) become invasive (Colautti and MacIsaac, 2004; Colautti *et al*., 2006). Propagule pressure can be divided into propagule size - the number of individuals introduced in a single release event and propagule number - the number of release events. As the number of individuals and the number of release events increase so too does the propagule pressure. Propagule pressure theory requires us to think about the invasion success of alien populations rather than the success of alien species (Lockwood *et al*., 2005).

Increased propagule pressure is thought to increase invasion success due to several factors, for example: 1) a wider geographic spread of a would-be-invasive reduces the chance of overall loss due to stochastic events; 2) genetic variation associated with increased propagule pressure can increase the chances of successful adaptation to novel selection pressures; 3) wide geographic spread increases the chances of individuals 'finding' a suitable habitat for establishment; and 4) large/consistent introductions of individuals enable the resident individuals to overcome problems generally associated with small populations (Lockwood *et al*., 2005).

As already mentioned there may be more species introduced into islands in the first place but so too may there be more propagule pressure on islands which might be a contributory factor for invasion success on islands. For example Daehler (2006) considers the high propagule pressure found in Hawai'i to account for about half the higher invasibility as compared to that found in Taiwan.

A greater understanding of the importance of propagule pressure on islands could be important to help reduce future invasions. For example invertebrate introductions are mostly accidental and understanding propagule pressure could lead to a better understanding of future quarantine requirements for islands. For plant invasions, preventing further introductions of existing populations of seemingly benign naturalised plants might also be important.

Some useful lessons could also be learned from propagule pressure theory and invasion biology in the field of habitat restoration. Habitat restoration aims to restore degraded or lost habitats to a similar state to their original species assemblage. Habitat restoration is fast becoming important for the maintenance of biodiversity in the wild and the logical outcome of spending large amounts of public funds on seed banks for wild species. Species introduction success rates could be improved by increasing propagule pressure by multiple introductions.

The theory of propagule pressure can also have implications for conservation biology.1) stressing the importance of maintaining several subpopulations of individuals and 2) that with a declining population there is a size under which extinction is almost certain. The provision of corridors that link patch habitats with larger ‘source’ patch, although often contentious, is thought to reduce the long term effects of inbreeding depression (Falcy and Estades, 2007).

Conclusions

Most authors agree that islands are more vulnerable to invasion than mainland areas (Herben, 2005). A central tenet of invasion biology on islands as proposed by Elton (1958) was that islands were more vulnerable due to their being more species-poor and that species richness provided resistance to invasion. It is now thought that it is more likely that the size of the species pools rather than species richness that contributes to giving islands their vulnerability to invasions (Herben, 2005).

It is likely that there is more than one reason that islands have increased vulnerability to invasions - increased opportunity i.e., more species are

introduced in the first place and increased propagule pressure and this has been found to improve invasion success (Lockwood *et al*., 2005). Increased disturbance on islands provides the naturalised/invasive plants with opportunities to exploit increased resource availability. The native species, often with their characteristic loss of dispersal mechanisms (Carlquist, 1974), might be slower to take advantage of this. Islands might currently also be undergoing increased disturbance due to improved mechanisation and increased levels of tourism. The fact that islands are by their definition geographically smaller than continental areas means that where habitats vary on islands there will be less distance between these different habitat types increasing chances of naturalised/invasive individuals 'finding' suitable habitats (Gimeno *et al.,* 2006).

Finally, the enemy release hypothesis (Keane and Crawely, 2002) likely contributes to the suite of characteristics that mean that islands are more vulnerable to invasive species.

It is thought that the islands that are most effected by invasive species are those that have had the least contact with humans prior to human colonisation – the Galápagos Islands could be a good example of this as they have only been settled relatively recently (Kühn and Klotz, 2007).

Research into invasive plant species on islands can also help us better understand the effects of habitat fragmentation in general; where mainland habitats can become more like islands - small, depauperate and disturbed habitats with missing groups of species (Denslow, 2003).

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Chapter 2

Taxonomy of tomatoes in the Galápagos Islands: native and introduced species of *Solanum* section *Lycopersicon* (Solanaceae)

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Taxonomy of tomatoes in the Galápagos Islands: native and introduced species of *Solanum* section *Lycopersicon* (Solanaceae)

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Contents

Abstract The Galápagos Islands are of great conservation interest due to their high proportion of endemic species. The endemic tomatoes (*Solanum* section *Lycopersicon*) of the islands have long been of interest to plant breeders. We analyse the morphology of all the Galápagos tomatoes: two endemic species, *Solanum cheesmaniae* and *S. galapagense* (the latter described here as new) and two introduced species, *S. lycopersicum* and *S. pimpinellifolium*. Morphological characters were measured on greenhouse-grown plants raised from seeds obtained from the wild and seed-bank accessions. Species boundaries were examined by cluster analysis and principal component analysis. Although the four taxa are distinct and therefore regarded as bona fide species they exhibit considerable intraspecific variation. A taxonomic treatment of the tomatoes in the Galápagos is provided, with keys to all solanums in the islands, descriptions, listings of representative specimens examined and full exsiccatae. Field observations of plants in the wild in the Galápagos are also included in the species descriptions. We highlight the potential for genetic contamination of the endemic tomatoes by hybridization and introgression with the two introduced species.

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Key words Ecuador, endemism, invasive species, Galápagos Islands, morphology, new taxa, Solanaceae, *Solanum*, species delimitation, tomato

Introduction

The flowering plant family Solanaceae contains many taxa of importance for humans, in agriculture (potatoes, tomatoes, peppers), medicine (mandrake, tobacco, deadly nightshade, henbane), and as ornamentals (*Solanum* spp., tobaccos, petunias). Members of the family occur worldwide, but the highest diversity, of both genera and species is found in the Neotropics. The largest genus in the family is *Solanum*, an estimated 1500–2000 species. This diversity places *Solanum* among the most species-rich genera of angiosperms. Species of *Solanum* exhibit an incredible range of morphological forms, ranging from tiny herbs to medium sized forest trees, and are found in all habitats worldwide. Species richness in *Solanum* (and in the family as a whole) is highest in the Neotropics, particularly in the Andes and associated valley systems.

Traditionally, several genera of economic importance have been segregated from *Solanum*, based largely on their use as human food plants. The tree tomatoes (*Cyphomandra* Sendtn.) and the tomatoes (*Lycopersicon* Mill.) have been shown to be deeply nested within a monophyletic larger *Solanum*, using both morphological (Child, 1990; Spooner *et al.*, 1993; Bohs, 1994, 1995) and molecular (Spooner *et al.*, 1993; Bohs & Olmstead, 1997, 1999; Peralta & Spooner, 2001) character sets.

As part of a larger study on the taxonomy and phylogeny of the wild tomatoes and their close relatives (Peralta *et al.*, in prep.) and broader studies of evolutionary genetics of the introduced and native tomatoes in the Galápagos (SD), we identified more variation between the Galápagos taxa than had been recognized previously (but see Lundgren *et al.*, 1985). This work is the result of field studies undertaken by SD, examination of herbarium specimens (see 'Plant specimens' and 'Taxonomic treatment') and morphometric analyses undertaken on greenhouse-grown accessions of all Galápagos tomato taxa. Our aims here are to document the variation found amongst and between Galápagos tomatoes, both native and introduced, to describe the species of tomatoes occurring in the islands and to provide tools for their identification that will be useful to those working with Galápagos plants.

History of tomato classification

Linnaeus (1753) described three species of what are now recognized as tomatoes as members of the genus *Solanum* (*S. lycopersicum*, *S. peruvianum* L. and *S. pimpinellifolium*). Philip Miller (1754), a contemporary of Linnaeus, segregated the new genus *Lycopersicon* to accommodate *Solanum* species with multi-locular fruits, including the tomatoes, the potato (*S. tuberosum* L.) and several other species (Miller, 1754; Peralta *et al.*, in prep.).

Use of the generic name *Lycopersicon* settled upon the relatives of the cultivated tomato (*Solanum lycopersicum*), that possessed yellow flowers, apparently longitudinal anther dehiscence and a long sterile appendage on the tips of the anthers (Peralta & Spooner, 2000). Some species in the group, however, are problematic with respect to this restricted generic definition. *Solanum pennellii* Correll, a species of the Peruvian and Chilean coastal deserts, has the requisite yellow flowers and apparently longitudinal dehiscence, but lacks the sterile anther appendage (Correll, 1962).

The classification of tomatoes as the genus *Lycopersicon* was maintained by several classical and modern botanists (Dunal, 1813, 1852; Müller, 1940; Luckwill, 1943; Correll, 1962; Symon, 1981; D'Arcy, 1991; Nee, 1999; Hunziker, 2001). Plant breeders have consistently maintained usage as *Lycopersicon* (Taylor, 1986; Rick, 1979, 1988; Rick *et al.*, 1990; C. M. Rick Tomato Genetics Resource Center. http://tgrc.ucdavis.edu). This treatment, however, has not been universal. In his original treatment of these species, Linnaeus (1753) included them as members of the genus *Solanum*, and many subsequent taxonomists have also recognized the tomatoes as belonging to *Solanum* rather than as the segregated *Lycopersicon* (Wettstein, 1891; Macbride, 1962; Seithe, 1962; Fosberg, 1987; Child, 1990).

Recent studies, firstly using morphological characters (Child, 1990) and then using molecular data from both the chloroplast and nuclear genomes (Spooner *et al.*, 1993; Bohs & Olmstead, 1997, 1999; Peralta & Spooner, 2001), have shown that the tomatoes are the sister group of the potatoes. If classifications are to be based on monophyletic groups (Judd *et al.*, 1999), the tomatoes and their relatives should be taxonomically treated as part of the genus *Solanum*. We are treating the Galápagos species examined here as species of *Solanum*, recognizing that the agronomy and plant breeding community might, for purely practical reasons, prefer to maintain familiar names of these taxa under the genus *Lycopersicon*. Nomenclatural issues arising from this will be treated in detail in an upcoming monograph of the wild tomatoes and their relatives (Peralta *et al.*, in prep.) and have also been examined previously (Spooner *et al.*, 1993).

Introduction to the Galápagos

The Galápagos Islands are volcanic in origin and straddle the equator about 1000 km to the west of the coast of South America (Republic of Ecuador), the nearest landmass. The oldest islands towards the east of the archipelago are between 2–6.5 million years old (Geist, 1996). The younger, more western islands still have considerable volcanic activity; seven eruptions occurred on Fernandina between 1958 and 1998, and Volcán Cerro Azul on Isabela erupted in 1979 and 1998 (Stephenson, 2000). There are 13 large islands (over 10 km^2) and over 40 officially named smaller islands, islets and emergent rocks (Fig. 1). The total landmass of the Galápagos is about 8000 km^2 (Jackson, 1993).

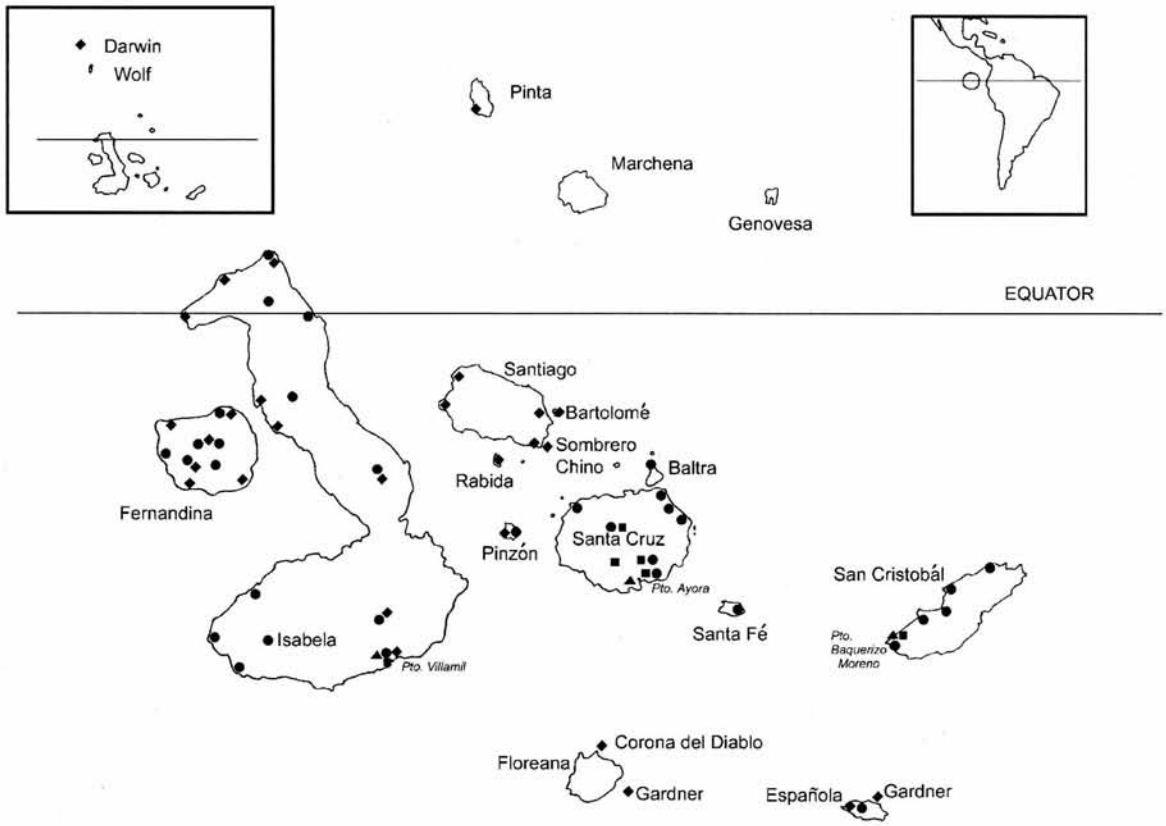


Figure 1 Distribution map of tomatoes in the Galápagos Islands (island names follow Table 1). *Solanum cheesmaniae*, circles; *Solanum galapagense*, rhomboids; *Solanum pimpinellifolium*, squares; *Solanum lycopersicum*, triangles.

The fourth Bishop of Panamá, Fray Tomás de Berlanga, discovered the islands in 1535, when his ship was becalmed en route from Panamá to South America. Pre-conquest discovery of the islands remains in dispute, despite the presence of fragments of pre-Columbian pottery and legends of Incas visiting the islands in the 1400s (Perry, 1972).

In the sixteenth century the islands were only periodically visited by buccaneers and adventurers and by whaling ships in the seventeenth and eighteenth century. The Galápagos Islands were used as a source of fresh food and water – particularly giant tortoises, which were easily captured and stored live on ship for up to a year (Perry, 1972).

The first 'proper' Galápagos scientific collections were made at the end of the eighteenth century by Archibald Menzies, a surgeon–naturalist collecting plants for Sir Joseph Banks and the Royal Botanic Gardens at Kew. He collected 'a few' plant specimens, which later were accidentally mixed with plants collected from Hawaii. David Douglas (collecting for what is now the Royal Horticultural Society), John Scouler and James McRae visited the islands on their way to the western coast of the United States and Canada in the late 1820s. Hugh Cuming, an independent collector of natural history specimens resident in Chile, visited the Galápagos in 1829 as part of his general exploration of the western South America. Their plant collections, combined with those collected by Charles Darwin in 1835, when the *Beagle* visited the islands, were the material of Joseph D. Hooker's enumeration of Galápagos plants eventually published in 1847 (see note under *S. cheesmaniae* for discussion of dates of publication). The fact that the *Beagle* and Darwin visited the Galápagos at all was due to several factors. First, British naval captains had been interested in obtaining good navigational charts of the islands. Second, reports of the geology and unknown species to be found there sparked interest in further exploration and third, William J. Hooker, then Professor of Botany at Glasgow but later knighted and director of the Royal Botanic Gardens at Kew, specifically lobbied for more Galápagos collections (Larson, 2001). The voyage of the *Beagle*, including the collections and observations made in Galápagos, was a turning point in Charles Darwin's career, and has made the islands a key locality not only for biodiversity, but for the history of science.

In 1832 the Galápagos Islands became a province of Ecuador, where they are officially known as the Archipélago de Colón. The individual islands have been known by a wide variety of names, both English and Spanish. Table 1 summarizes the island synonyms relevant here. We use the official Ecuadorian island names (W. Tapia Aguilera, pers. comm., 2002) for discussion and in the specimen citations.

There are now five islands with permanent human habitations – Floreana, San Cristóbal, Isabela, Santa Cruz and Baltra. The first island to be settled was Floreana, in

Official island and islet names	Synonyms
Baltra	South Seymour
Bartolomé	Bartholomew
Corona del Diablo (near Floreana)	Devil's Crown; Onslow
Darwin	Culpepper
Española	Hood
Fernandina	Narborough
Floreana	Charles; Santa María
Gardner (near Española)	
Gardner (near Floreana)	
Isabela	Albemarle
Pinta	Abingdon
Pinzón	Duncan
Rabida	Jervis
San Cristóbal	Chatham
Santa Cruz	Indefatigable
Santa Fé	Barrington
Santiago	James; San Salvador
Sombrero Chino	
Wolf	Wenman

Table 1 Galápagos Island names used in this study and synonyms (Slevin, 1959; W. Tapia Aguilera, pers. comm., 2002).

about 1807, by a marooned crew-member of a British ship (Slevin, 1959). In the late 1800s San Cristóbal and Isabela were settled, the latter with two villages established: Villamil on the south coast and San Tomás, to the north of Villamil. In 1926 Santa Cruz was settled by a group of Norwegian farmers, and finally Baltra in the 1940s became a US airbase (it is now an Ecuadorian Naval base) (Jackson, 1993). Santiago, in the past, had a settlement at Bahía James (Wiggins & Porter, 1971). The last published census in 1999 estimated a permanent resident population of just over 16 000 (http://naturalist.net/news/Galápagoscensus.html).

Most of the landmass and the waters surrounding the Galápagos are now officially protected. In 1959 the Ecuadorian government designated the uncolonized areas, approximately 90% of the landmass, as a Galápagos National Park. In the same year the Charles Darwin Foundation was set up dedicated to the conservation of the Galápagos ecosystems and a year later the Charles Darwin Research Station (CDRS) was established on Santa Cruz to conduct scientific research and environmental education (http://www.darwinfoundation.org). The islands were designated a UNESCO World Heritage Site in 1978. In 1986 the waters between the islands and 15 nautical miles (extended later to 40 nautical miles) surrounding the islands were designated as the Galápagos Marine Resources Reserve by INEFAN (Instituto Nacional Ecuatoriano de Fauna y Areas Naturales). In 2001 the Galápagos Marine Reserve was also designated a UNESCO World Heritage Site (http://whc.unesco.org/nwhc/pages/sites/main.htm).

Vegetation types vary within and between the islands and are dependent on several factors, including the age and size of the island, altitude and orientation. The younger islands have lava flows so recent that they have not fully been colonized by plants (Wiggins & Porter, 1971). The small low islands such as Baltra rarely receive much rain. Areas at higher elevations and on southern aspects receive precipitation from the prevailing southerly winds. On Santa Cruz, for example, the lowland arid zone receives between 0–300 mm of rain per annum; above this there is a moist zone receiving up to 1700 mm of rain per annum where cloud forest occurs between about 300–700 m altitude (Jackson, 1993).

Darwin wrote, soon after arriving in the Galápagos, 'I certainly recognize S. America in Ornithology. Would a botanist? 3/4 of the plants in flower' (quoted in Keynes, 1988). It is now documented that about 40% of the native flowering plant taxa are endemic to the islands (Wiggins & Porter, 1971). The total native vascular plant diversity in the Galápagos numbers approximately 596 taxa; with 372 natives and 224 endemics (Lawesson, 1990*b*). In common with other oceanic archipelagos, the Galápagos Islands suffer from the effects of introduced animals and plants (Loope *et al.*, 1988) brought in either accidentally or intentionally by humans. Fifteen years ago, the number of introduced plants in Galápagos was estimated to be approximately 260 (Lawesson, 1990*a*). More recent analyses show that the number of introduced plants is rising, currently it stands at over 500 taxa; 260 of these are naturalized in the islands (A. Tye, pers. comm. in prep., 2002).

History of tomato classification in the Galápagos

The earliest collection of any of the endemic tomato species is that of John Scouler (*Solanum galapagense*, *Scouler s.n.*, E), collected in 1827. Both species of endemic Galápagos tomatoes were collected by Charles Darwin in 1835. Along with Darwin's other botanical collections they were described by Joseph D. Hooker, from specimens sent to him via John Stevens Henslow from the University of Cambridge (Porter, 1980). Hooker (1847) recognized three types of Galápagos tomatoes, all of them similar to specimens found in the mainland of South America: '*Lycopersicon pimpinellifolium* – Chatham Island' (San Cristóbal) ('precisely similar to the South American plant'), '*Lycopersicon esculentum* var. *minor* – James Island' (Santiago) (being 'smaller than the common state of the species') and '*L. peruanum* var. *parviflorum* – Chatham Island' ('having smaller flowers than its mainland counterpart and no other difference seems to exist').

Andersson (1855) described the Galápagos tomatoes using both his own collections from the islands and Darwin's, but did not attempt to reconcile the already complex nomenclature of the Galápagos plants. He included three species in his treatment, '*Lycopersicum esculentum*', '*Lycopersicum pimpinellifolium*' and '*Lycopersicum peruvianum*', the latter with two informally named variants: '*a*' – 'a procumbent plant with irregularly toothed leaflet margins and subappressed pubescence' from Isabela and '*b*' – 'an erect plant with deeply divided leaf segments and long, divaricate hairs' from San Cristóbal. Robinson's (1902) *Flora of the Galápagos Islands* identified four taxa of '*Lycopersicum*', in general the same

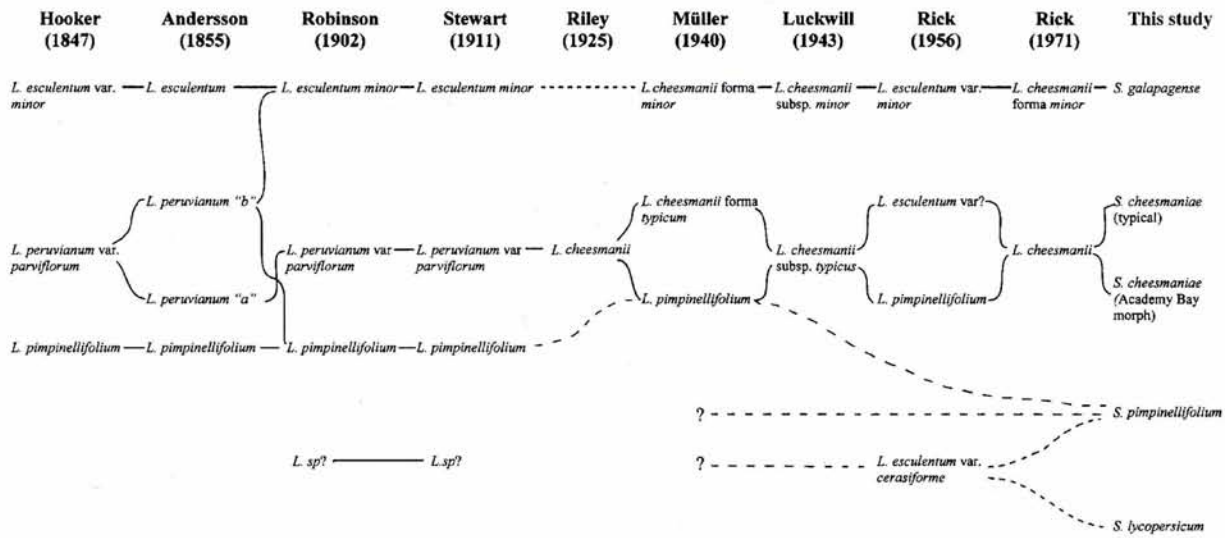


Figure 2 History of classification of tomatoes in the Galápagos.

as those recognized by Andersson, with the exception of an unnamed species from San Cristóbal which he identified as lacking the spreading pubescence of the other Galápagos tomatoes. Robinson (1902) recognized '*Lycopersicum esculentum* var. *minor*' as distinct, not relegating it to synonymy as had Andersson (1855). In the California Academy's botanical survey of the islands, Stewart (1911) followed Robinson (1902), maintaining the same four taxa of tomatoes in the Galápagos (Fig. 2).

Riley (1925) was the first to recognize the species-level distinctness of the Galápagos tomato and he described it as '*Lycopersicon cheesmanii*' (based on Evelyn Cheesman's specimen collected from Santa Cruz). Riley mentioned Andersson's collections and descriptions of the two variants of '*L. peruvianum*' from Galápagos, but stated that his new species differed from both.

In the 1940s, two herbarium taxonomists revised the tomatoes (as *Lycopersicon*) throughout their ranges. Müller's (1940) revision recognized three taxa occurring in the islands: '*L. cheesmanii* forma *minor*', '*L. cheesmanii* forma *typicum*' and '*L. pimpinellifolium*'. He placed all of the Galápagos tomatoes except those with larger leaves into a single species with two forms. The revision by Luckwill (1943) recognized only two tomato taxa from the Galápagos: '*L. cheesmanii* subspecies *typicus*' and 'subspecies *minor*'. Luckwill's (1943) circumscription of his '*L. pimpinellifolium*' did not include any of the specimens identified as that species occurring in Galápagos, but he indicated that its occurrence on the Galápagos needed confirmation. Both of these revisions were completed during the Second World War, a time difficult for the loan of specimens and for travel to the herbaria of Europe, where many of the previously collected specimens of these plants were held.

Since the 1950s, interest has increased in wild species as sources of genes for the improvement of the cultivated tomato. This interest was reflected in the increased collecting intensity for breeding purposes, with germplasm being kept for use by all breeders in central localities. Collections of Galápagos tomatoes held in the C. M. Rick Tomato Genetics Resource Center (TGRC: http://tgrc.ucdavis.edu) seed bank, Department of Vegetable Crops, University of California, Davis, were described by Rick (1956), who collected extensively on the Galápagos Islands in the 1950s and 1960s. He recognized three taxa: '*L. pimpinellifolium*' (TGRC accession number LA166), '*Lycopersicon esculentum* var. *minor*' (TGRC accession number LA317), and '*L. esculentum* var. *cerasiforme*' (TGRC accession number LA292); the latter he later assumed had been introduced by human settlers (Rick, 1963). He also briefly mentioned another less widely distributed form of tomato as a variation of '*L. esculentum*' – we believe that he was referring to the 'typical' form of *Solanum cheesmaniae*, as exemplified by the type specimen *Cheesman in Riley 403*.

In the *Flora of the Galápagos Islands* (Wiggins & Porter, 1971), Rick (1971) recognized only two taxa of Galápagos tomatoes: '*Lycopersicon cheesmanii*' (combining the taxa previously recognized as '*L. pimpinellifolium*', '*L. cheesmanii* forma *typicum*' and the briefly mentioned variation of '*L. esculentum*') and '*L. cheesmanii* forma *minor*'. This circumscription of the Galápagos tomatoes has been maintained (Rick, 1983) and is the current nomenclature used at the TGRC and thus throughout the plant breeding community.

Distribution and ecology of tomatoes in the Galápagos

Tomatoes occur on 19 different islands and islets throughout the archipelago (Fig. 1). On only two, Fernandina and Isabela, do the two endemic taxa appear to grow sympatrically in time as well as space. During the course of this research we found the two introduced tomatoes (*Solanum pimpinellifolium* and *S. lycopersicum*) on Santa Cruz, Isabela and San Cristóbal.

These three islands also support populations of endemic tomatoes, and the introduced tomatoes are now found in localities where the native tomatoes were collected in the past.

Both of the native Galápagos tomato species always develop yellow to orange ripe fruit; no native biotype has bright red fruit. This was also the opinion of the late Charles M. Rick, who considered all tomatoes with red fruit to be introduced taxa – either *Solanum pimpinellifolium* or *S. lycopersicum* (C. M. Rick, in litt., 1998). The specimen described by Hooker as '*L. pimpinellifolium*' based on Darwin's 1835 collections does not match the Linnaean type specimen and is clearly not the same species as the mainland *S. pimpinellifolium* with bright red fruit (Peralta *et al.*, in prep.; also see 'Taxonomic treatment'). To further complicate matters, genuine plants of *S. pimpinellifolium* and *S. lycopersicum* appear to have been introduced by human settlers from the mainland to Galápagos during the twentieth century.

The occurrence of putatively feral plants of *Solanum lycopersicum* (as *Lycopersicon esculentum* var. *cerasiforme*) on Isla San Cristóbal has been documented since the 1950s (Rick, 1956). *Solanum lycopersicum* was collected on San Cristóbal (*Howell 8573*) in 1932, from Santa Cruz in 1952 (TGRC LA 0292) and in 2000 on Isabela (*S. Darwin 302*). *Solanum pimpinellifolium* (as *L. pimpinellifolium*) has also been recorded in the archipelago (Rick, in litt., 1998). The earliest unequivocal collection was made in 1985 on Isabela (TGRC LA 2857). However it is possible that *S. pimpinellifolium* has occurred in the islands a lot longer. Müller (1940) cited several Galápagos specimens as *S. pimpinellifolium*; most belong to a variant of *S. cheesmaniae* informally recognized here as the 'Academy Bay' morph, save one (*Stewart 3380*). The leaf morphology of this specimen fits extremely well with the Linnaean type of *S. pimpinellifolium*. It is also similar to plants of *S. pimpinellifolium* currently found in the Galápagos, however, this specimen lacks mature fruit, precluding a firm identification (see 'Taxonomic treatment'). *Solanum pimpinellifolium* has been collected more recently in 2000 on Santa Cruz (*S. Darwin 103*) and San Cristóbal (*S. Darwin 278*).

Rick & Bowman (1961) found that the endemic tomatoes had severe seed dormancy broken by passage through the gut of the Galápagos giant tortoise (*Geochelone elephantopus*). Fewer than 1% of untreated seeds germinated (Rick & Bowman, 1961). However, populations of the endemic tomatoes occur in many areas within the archipelago that either no longer have, or possibly never had, resident populations of giant tortoises (Bartolomé, Sombrero Chino, Corona del Diablo and Darwin). The endemic tomatoes are known to be early colonizers of recent lava flows (Fosberg, 1987). Dispersal of seeds in salt water seems unlikely because exposure of seeds of the endemic tomatoes to even 20% salt water was shown to reduce seed viability (Kurth *et al.*, 1986). Rick & Bowman (1961) suggested that mockingbirds (*Nesomimus parvulus parvulus*), iguanas or feral goats were also potential seed dispersers of the endemic tomatoes. Dispersal mode and germination promoters of the Galápagos tomatoes in the wild have yet to be determined. Preliminary investigations (S. Darwin, unpubl. obs.) suggest that seed dormancy may be less strong than previously thought.

Morphological analyses

Plant specimens

In order to evaluate the morphological variation among species, we examined a large number of herbarium specimens from the Galápagos Islands (see Exsiccatae) and mainland South America, as well as plants that were grown from seed from wild and seed bank accessions and grown under greenhouse conditions. Valuable data about morphological variation among tomato populations and species in their natural habitats were obtained on Galápagos by SD. Tomatoes observed growing in the wild, greenhouse-grown plants and herbarium specimens were used to examine morphological variability and for the species descriptions but only greenhouse-grown plants were used for phenetic analysis. We have thereby examined a wider range of specimens than in any other previous treatment of these species.

Specimens examined were loaned by herbaria cited in the text, following the conventional abbreviations of Holmgren *et al.* (1990). The complete database of herbarium specimens of both wild and cultivated material examined is available from SK on request. Vouchers for all material grown are deposited at BM and CDS.

The comparative morphological study of 186 individuals from 84 accessions (on average two plants per accession) was performed using plants grown under heated greenhouse conditions at the Chelsea Physic Garden, London between November 2000 and March 2001. Plants were grown from both wild-collected seed accessions (SD in 2000) and from seed accessions kindly provided by Dr Charles Rick and Dr Roger Chetelat from the TGRC. The accessions included in this research represent much of the tomato diversity that exists throughout the archipelago.

Seed dormancy was broken using the method recommended by Rick & Borgnino (http://tgrc.ucdavis.edu/seed_germ.htm). The dry seed weight was measured prior to treatment, and this character was included in the morphometric analyses. The seeds were soaked in 2.7% sodium hypochlorite for 30 minutes, then rinsed in fresh water and placed on moist blotting paper in numbered Petri dishes and stored in the dark. This treatment was repeated after 10 days to any un-germinated seeds. The seedlings were then transferred to 80 mm welled seed trays in John Innes loam (seven parts loam, three parts coir and two parts grit and bark). The trays were placed in a heated greenhouse. On average, seeds of *Solanum pimpinellifolium* germinated in approximately 11 days and those of the endemic species in approximately 20 days. As the experiments were conducted in the British winter, additional artificial lighting was provided between 10.00–12.00 h and between 16.00–18.00 h. Measurements were made on mature, reproductive plants.

Characters used

For statistical and phenetic analyses, a total of 49 characters were assessed (45 quantitative and four qualitative). Characters were selected from Rick (1983), Peralta (2000) and from personal observations. The quantitative characters included 19 ratios that assessed shapes of different plant organs

(Table 2). Both original characters and ratios are presented in Table 2.

Data analysis

The mean, range and standard deviation were estimated for each character within each putative species using SYSTAT (1999). ANOVA was performed (also in SYSTAT, 1999) on all continuous quantitative characters and all accessions to evaluate significant differences between taxa ($P \leq 0.005$).

For the phenetic analysis we considered that the operational taxonomic unit (OTU) was the 'locality group' presented in Table 3. The 22 locality groups are based on the accession passport in the TGRC seed bank and from the collection notes made by SD in the Galápagos. Where the localities of the TGRC and the wild collections overlap they are considered as the same locality group (Table 3). Some locality groups are represented by only a single accession because many Galápagos populations are small, and can consist of only a single plant. We considered that the OTUs represented natural groups, for the clarity of the interpretation an average was therefore calculated to represent each OTU.

Cluster analyses were produced by NTSYS-pc^R version 2.0 (Rohlf, 1992) using 33 characters, 23 continuous quantitative characters including 12 ratios, six discontinuous quantitative characters and three binary and one multistate qualitative character. The mean of each character was used for the phenetic analyses, and for that reason only the four qualitative characters can be assumed to be linear and thus treated as quantitative data (Abbott *et al.*, 1985). Averages for each character were standardized (STAND) and similarity matrices were generated, using average taxonomic distance (DIST), Manhattan distance (MANHAT) and Euclidean distance (EUCLID). Clustering was performed using the unweighted pair-group method (UPGMA) in SAHN. Cophenetic correlation coefficients (COPH and MXCOMP) were used to measure distortion between the similarity matrices and the resultant three phenograms (Rohlf & Sokal, 1981; Sokal, 1986). Principal component analyses (PCA) were performed on standardized data also using NTSYS. PCA makes no assumptions about group membership of OTUs under analysis, and effectively portrays the variation present in the data.

Results and discussion

The phenetic analyses based on morphological characters support the circumscription of four distinct species of tomatoes currently occurring in the Galápagos Islands: *Solanum cheesmaniae* and *S. galapagense* (endemic) and *S. pimpinellifolium* and *S. lycopersicum* (introduced).

The ANOVA test showed significant differences among 30 characters or character ratios scored for all taxa (Table 2). For *Solanum galapagense*, leaf characters were found to be most distinctive and most strongly statistically supported (Table 4). Leaf structure in *S. galapagense* is more complex than in any other tomato species; the presence of more primary, secondary and interjected leaflets differentiate it from the other three species. *Solanum galapagense* usually also has tertiary leaflets and occasionally quaternary lobing (very occasionally to leaflets), and this level of leaf division has not been observed in herbarium or cultivated accessions of the other three taxa during this research. Flower characters were significantly different for *S. pimpinellifolium* which has longer, more lanceolate corolla lobes than the other species.

Similar dendrograms were produced by DIST (Fig. 3A), EUCLID and MANHAT coefficients, and the OTUs clustered in four groups that correspond to the four previously recognized tomato entities from in the Galápagos, which we here recognize at the specific level. The cophenetic correlation is 0.77 when the first two coefficients were used, and 0.78 for the third. These values are good fits (almost good fits *sensu* Rohlf, 1992) to the cluster analysis.

Principal component analysis showed a similar relationship among OTUs as did the cluster analysis (Fig. 3B). The three principal components explained almost 70% of the variation found in the data set (first 37.6%, second 19.4% and third 11%). A further PCA performed in a subset of leaf characters (not illustrated) showed only plants of *S. galapagense* as distinct from the other taxa.

Solanum galapagense and *S. cheesmaniae* can readily be differentiated from *S. pimpinellifolium* and *S. lycopersicum* on fruit and seed characters and *S. pimpinellifolium* can be separated from the other three species by flower shape. *Solanum galapagense*, as has been recognized by most others studying Galápagos tomatoes (Fig. 2), is a markedly distinct taxon, which we recognize here at the specific level. The explicit morphological analyses on wild and cultivated plants, and complementary studies performed on herbarium specimens were very useful to show the relationships among taxa and to support our taxonomic treatment.

Studies of allozyme diversity in the Galápagos tomatoes support our species circumscription, revealing unique fixed allelic differences in several enzyme systems, particularly in *Solanum galapagense*. However, no fixed allelic differences between the different morphs within *S. cheesmaniae* were detected (Darwin *et al.*, in prep).

The origin of the Galápagos tomatoes remains unresolved. Phylogenetic analyses using DNA sequences of the nuclear gene *waxy* (GBSSI: granule-bound starch synthase), show that these four species discussed here are extremely closely related, suggesting recent origins and rapid morphological differentiation (Peralta & Spooner, 2001). Rick (1963) proposed that the closest relatives to the Galápagos tomatoes were *Solanum pimpinellifolium* and *S. habrochaites* S. Knapp & D.M. Spooner (as *Lycopersicon hirsutum* Dunal). Allozyme electrophoresis (Rick & Fobes, 1975) suggested that the populations of *S. pimpinellifolium* most closely related to the Galápagos tomatoes were from coastal Perú and that the most similar morphologically were populations from the region of Motupe-Olmos in the northern Peruvian department of Lambayeque (*c.* 6°S latitude). The Humboldt Current flows from here to the Galápagos at certain times of year giving support to this suggestion (Rick & Fobes, 1975).

Observations of the distribution and cover of the two introduced tomatoes were made in the field on Santa Cruz and Isabela to enable a classification of their status as alien plants.

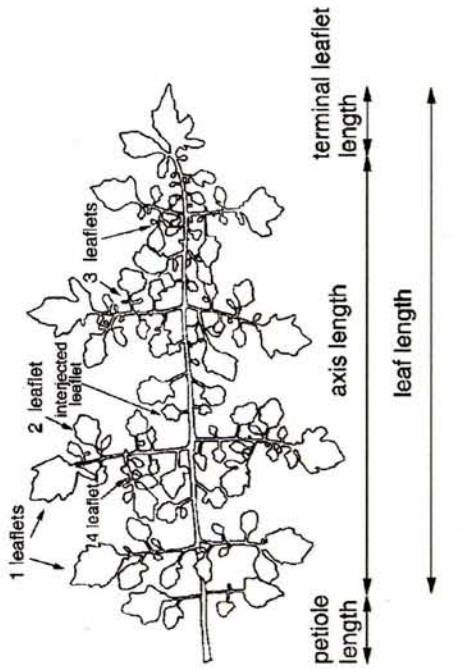
Characters	Descriptions
Leaves	
1. **Petiole length (mm)***	
2. **Leaf length (mm)***	
3. Leaf axis length (mm)*	
4. Length from widest to leaf apex (mm)*	
5. Leaf width (mm)*	
6. Terminal leaflet length (mm)	
7. **Number of primary leaflets**	
8. **Number of secondary leaflets**	
9. **Number of tertiary leaflets**	
10. **Number of interjected leaflets**	
11. **Leaf length/petiole length ratio***	
12. Leaf length/leaf axis length ratio*	
13. **Leaf length/length from widest to leaf apex ratio***	
14. **Leaf length/leaf width ratio***	
15. **Leaf length/terminal leaflet length ratio***	
16. Leaf axis length/petiole ratio*	
17. Leaf axis length/terminal leaflet length ratio*	
18. Length from widest to leaf apex/petiole ratio*	
19. Leaf width/leaf axis length ratio*	
20. **Leaf width/terminal leaflet length ratio***	
21. **Terminal leaflet length/petiole ratio***	
22. Terminal leaflet length/length from widest point to leaf apex ratio*	
Inflorescence	
23. Inflorescence axis length (mm)	
24. **Number of flowers per axis**	
25. **Number of branches**	
26. **Bract-like leaflets**	
27. **Bracteole-like leaflets**	
28. **Inflorescence axis length/number of flowers per axis ratio**	

Table 2 Characters measured for morphometric analysis. Characters used in the multivariate analysis are in bold, characters significantly different ($P \leq 0.005$) using the ANOVA test are marked with an asterisk.

Solanum lycopersicum was found in rubbish dumps and by roadsides extending a maximum of about 20 m away from human habitation. We would currently classify this species as a casual alien (*sensu* Richardson *et al.*, 2000). *Solanum pimpinellifolium* was also found in disturbed areas, rubbish dumps, roadsides and quarries, but it often occurs up to several km away from human habitation; for example along the roadside at Los Gemelos on Santa Cruz. It was also found at El Chato on Santa Cruz in an area seemingly undisturbed except for a tourist track and a population of giant tortoises. In this area the population of *S. pimpinellifolium* was so dense in places that it was virtually the only vascular plant present. We therefore classify *S. pimpinellifolium* preliminarily as an invasive plant (*sensu* Richardson *et al.*, 2000), and one which warrants further investigation to establish whether or not it is causing a negative impact on native species. Invasive plants can have many negative effects on native biodiversity. Many important food crops hybridize with their wild relatives in areas of sympatry (Ellstrand *et al.*, 1999) and there is a real potential in Galápagos for these introduced tomatoes to threaten the genetic integrity of the endemic tomatoes through hybridization and introgression.

There seem to be no barriers to hybridization of the different species of tomatoes in Galápagos (also see below). In the field, variously exserted styles were found in individuals of all four species, enabling pollen from another flower to be received on the stigma. The endemic carpenter bee (*Xylocopa darwini*) was observed (S. C. Darwin, unpubl. obs.) visiting flowers of *Solanum cheesmaniae, S. galapagense* and *S. pimpinellifolium* (in allopatry). Rick & Fobes (1975) found

Flowers	
29. **Length from centre of the corolla to apex of the corolla lobe** (mm) **A***	
30. **Length of the corolla lobe from apex to the corolla lobe junction** (mm) **B**	
31. **Length from centre of the corolla to the corolla lobe junction** (mm) **C***	
32. **Corolla lobe width** (mm) **D***	
33. **Total anther length** (mm) *	
34. Anther appendage length (mm) *	
35. **Style length** (mm) *	
36. **Style exsertion length** (mm)*	
37. **Pedicel length** (mm)	
38. **Pedicel articulation to axis length** (mm)	
39. **Sepal length** (mm)*	
40. Sepal width (mm)	
41. **Anthocyanin pigmentation on sepals**	
42. **Sepal angle on fruit**	appressed straight reflexed
43. **Length from centre of the corolla to apex of the corolla lobe/length from centre of the corolla to the corolla lobe junction ratio***	
44. **Length from centre of the corolla to apex of the corolla lobe/corolla lobe width ratio (A/C)***	
45. Length of the corolla lobe from apex to the corolla lobe junction/length from centre of the corolla to the corolla lobe junction ratio (A/D)*	
46. **Length of the corolla lobe from apex to the corolla lobe junction/corolla lobe width ratio (B/C)***	
47. **Total anther length/anther appendage length ratio (B/D)***	
48. **Sepal length/sepal width ratio***	
49. **Mean seed weight** (mg)*	

Table 2 Continued.

that there was 'little or no' insect activity and that the floral structure of the Galápagos tomatoes was adapted to automatic self-pollination (i.e. the styles were included within the staminal column). This is not consistent with our observations. In addition the discovery of sympatric populations of the endemic and the introduced species of tomatoes on Isabela highlight these concerns. The threat of hybridization between endemic and introduced tomatoes had already been suggested by A. Tye (in litt., 1999). In crossing experiments, Rick (1963) found that all four tomato species concerned here were fully intercompatible. Thus, hybridization and introgression could and might already be taking place between the four species.

Species concepts

In previous taxonomic treatments of the tomatoes (publications of C.M. Rick, TGRC), species circumscription largely followed the biological species concept (i.e. species being groups of interbreeding populations that are unable to interbreed with other such groups; Briggs & Walters, 1997). Rick (1963) found no barriers to crossing between the endemic Galápagos tomatoes; in fact, most species of tomatoes experience some degree of interpopulational geneflow, especially self-compatible populations (see Rick, 1979). Rick (1971) also observed individual plants on both Isabela and Fernandina that he considered to be morphological intermediates between

Island and locality names	OTU	*che*	*gal*	*pim*	*lyc*
Santa Cruz	5				
Punta Carrion	5a	X+ (1)			
Between Cerro Colorado and Punta Carrion	5b	X+ (13)			
North of Cerro Colorado	5c	X+ (4)			
New basura and quarry	5d			X+ (3)	
Los Gemelos	5e			X+ (7)	
El Chato Tortoise Reserve	5f			X+ (11)	
Academy Bay	5h	X* (5)		X*+ (3)	
Bella Vista and roadside	5g			X+ (1)	
Isabela	2				
Cabo Tortuga	2a		X* (2)		
Tagus Cove	2b		X* (1)		
Volcán Alcedo	2c		X* (1)		
San Tomás and basura	2d	X* (2)			X+ (6)
Road from Villamil to San Tomás	2e		X*+ (3)		
San Cristóbal	7				
Puerto Baquerizo Moreno	7a				X+ (2)
Santiago	3				
Bartolomé	3a		X*+ (11)		
Fernandina	1				
North side, low elevations	1a	X* (1)			
'Low elevations'	1b	X* (1)			
Volcán	1c	X* (3)			
Pinzón	4a		X* (1)		
Corona del Diablo	6a		X* (2)		

Table 3 Locality groups as OTUs for the accessions used in cluster and principal component analyses. A total of 186 plants were measured representing 84 accessions. * denotes TGRC collection, + denotes wild collection (SD 2000), numbers in parentheses denote number of accessions used. *che* – *S. cheesmaniae*, *gal* – *S. galapagense*, *pim* – *S. pimpinellifolium* and *lyc* – *S. lycopersicum*.

Character number	Character description	*che*	*gal*	*pim*	*lyc*
12	Leaf length:leaf axis length ratio	1.808 a	1.455 b	2.064 c	2.012 ac
13	Leaf length:length from widest to leaf apex ratio	1.317 a	1.495 b	1.312 a	1.359 a
14	Leaf length:leaf width ratio	1.199 a	1.292 b	1.121 a	1.179 a
15	Leaf length:terminal leaflet length ratio	2.422 a	3.495 b	2.031 a	2.098 a
16	Leaf axis length:petiole length ratio	2.231 a	3.764 b	2.725 a	2.338 a
17	Leaf axis length:terminal leaflet length ratio	1.422 a	2.495 b	1.031 a	1.098 a
18	Length from widest to leaf apex ratio:petiole length ratio	2.929 a	3.666 b	4.268 b	3.464 a
19	Leaf width:leaf axis length ratio	1.350 a	1.000 b	1.586 c	1.533 a
20	Leaf width:terminal leaflet length ratio	2.007 a	2.749 b	1.827 a	1.819 a
21	Terminal leaflet length:petiole length ratio	1.668 a	1.618 a	2.891 b	2.209 b
22	Terminal leaflet:length from widest to leaf apex ratio	0.565 a	0.447 b	0.661 a	0.673 a

Table 4 Means of leaf characters ratios that were found to be statistically significantly different ($P \leq 0.005$) between the taxa, and are indicated with a unique letter. The boxed regions indicate the characters that statistically support *Solanum galapagense* (see Table 2 for character descriptions). *che* – *S. cheesmaniae*, *gal* – *S. galapagense*, *pim* – *S. pimpinellifolium*, *lyc* – *S. lycopersicum*.

Solanum cheesmaniae and *S. galapagense* (also see discussion of *S. cheesmaniae*, p. **41**). He concluded that the two endemic Galápagos tomato taxa should be recognized as two forms of one species due to their ability to interbreed (Rick, 1971).

In contrast, our views on species delimitation basically follow what is known as the 'morphological cluster' species concept (Mallet, 1995): i.e. 'assemblages of individuals with morphological features in common and separate from other such assemblages by correlated morphological discontinuities

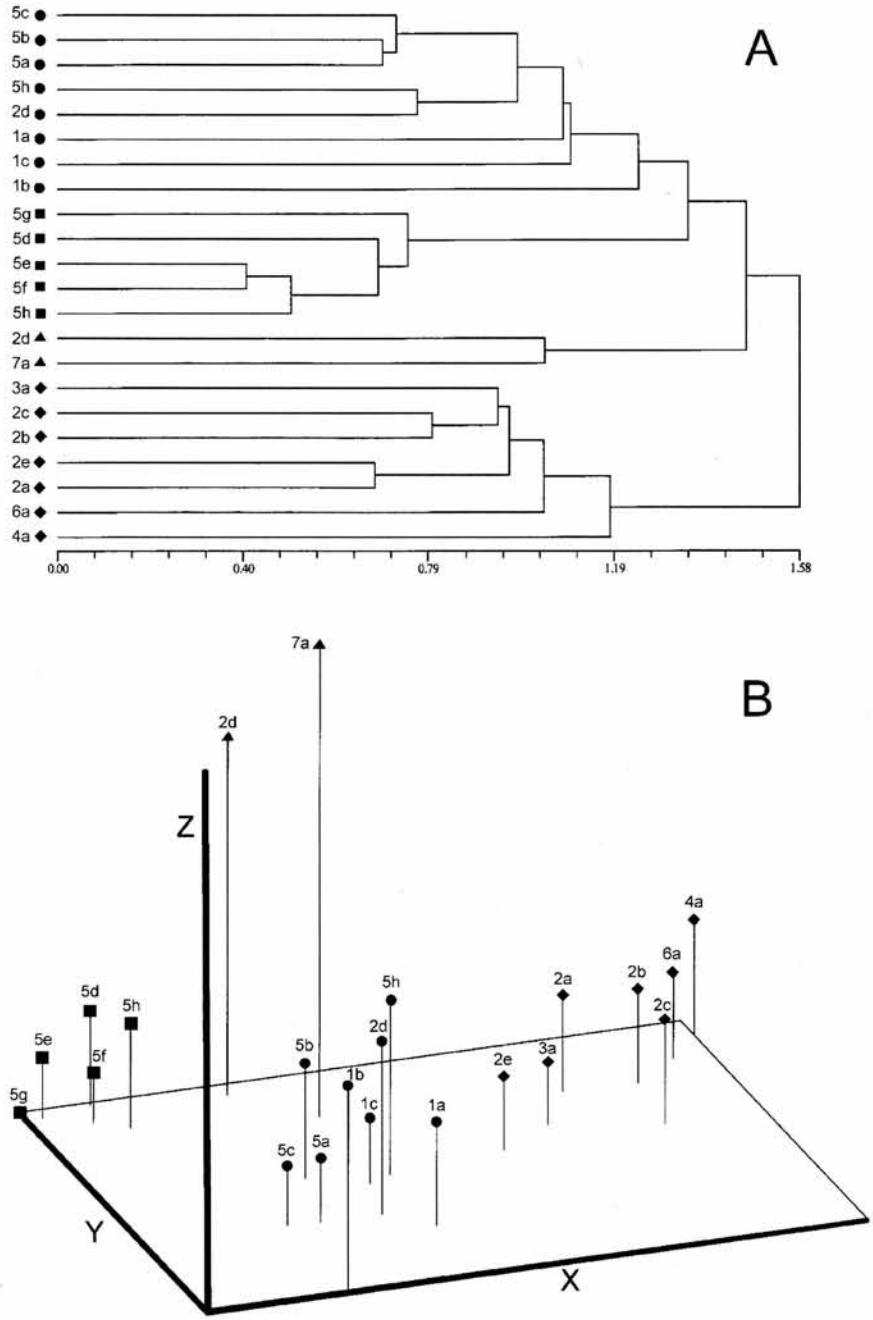


Figure 3 Results of the morphological analyses. A. Average taxonomic distance coefficient dendrogram. B. Principal component analysis (x – 1°, y – 2°, z – 3°): *Solanum cheesmaniae*, circles; *Solanum galapagense*, rhomboids; *Solanum pimpinellifolium*, squares; *Solanum lycopersicum*, triangles. See text for details.

in a number of features' (Davis & Heywood, 1963). Biological (Mayr, 1982), phylogenetic (Cracraft, 1989) and the host of other finely defined species concepts (see Mallet, 1995) are almost impossible to apply in practice when dealing with complex, highly variable groups and are therefore of little utility in a practical sense. It is important however to clearly state the criteria for the delimitation of species, rather than dogmatically follow particular ideological lines (see Luckow, 1995; Davis, 1997). We have been conservative in our approach, recognizing as distinct entities those population systems (sets of specimens) that differ in several morphological characteristics or in combinations of these characteristics. We have not formally recognized subspecific categories, although we have described and documented the variation where it occurs. *Solanum cheesmaniae* includes two marked variants, which we here describe as morphs of a variable entity. The patterns of variation and the presence of intermediates between these two entities are such that no reliable units can be extracted, and we prefer to not encumber the literature with excess names at present. We have described the variation, realizing that other

taxonomists may wish to interpret it differently; future study of this variation is underway (see p. **41**).

Taxonomic treatment

Solanum section **Lycopersicon** (Mill.) Wettst. in Engl. & Prantl, *Natürlichen Pflanzenfamilien* IV, 3b (65): 24 (1891).

Lycopersicon Mill., *Gard. Dict.* ed. 4, abr. (1754). Lectotype species (designated by D'Arcy, 1972): *Lycopersicon esculentum* Mill. (1768). (= *Solanum lycopersicum* L.)

Amatula Medik., *Malvenfam.* 106 (1787). Type: *Amatula flava* Medik. (= *Solanum lycopersicum* L.).

Solanum section *Lycopersicon* (Tourn.) Bitter, *Botanische Jahrbücher* **54**: 500 (1917). Lectotype species (designated by D'Arcy, 1972): *Lycopersicon esculentum* Mill. (1768). (= *Solanum lycopersicum* L.)

Solanum subgenus *Lycopersicon* (Tourn.) Seithe, *Botanische Jahrbücher* **81**: 204 (1962). Lectotype species (designated by D'Arcy, 1972): *Lycopersicon esculentum* Mill. (1768). (= *Solanum lycopersicum* L.)

Perennial, biennial or annual herbs; branches usually sprawling or vining, robust to slender. Stems glabrous to variously pubescent, the trichomes always simple and usually uniseriate. Sympodial units di- or trifoliate (in Galápagos trifoliate only). Leaves interrupted imparipinnate, sometime with secondary and tertiary leaflet formation; estipulate, but occasionally with well-developed pseudostipules; leaflets variously lobed, the margins entire to coarsely dentate; petiole usually shorter than the leaf blade. Inflorescences simple to several branched, bracteate or ebracteate; peduncle present, the flowers never basal. Flowers actinomorphic or somewhat zygomorphic; calyx 5-parted, usually pubescent; corolla yellow, 5-parted, lobed to the base to about halfway to the base, the lobes deltate to lanceolate; stamens 5, usually coherent in a tube with or without an apical sterile tip; anthers with variously developed papillae laterally; ovary minutely glandular villous to densely pubescent; style as long as or longer than the staminal column, exserted or included; stigma minute to capitate. Fruit a globose berry, green to whitish or brightly coloured red, yellow or orange, usually 2-locular, but in cultivated species variously multi-locular; calyx in fruit accresent, the lobes shorter than or longer than the mature fruit; seeds lenticular, appearing densely hairy due to the elongate testa cell walls.

Species descriptions of the introduced species of tomatoes are here taken only from Galápagos collected specimens. Synonymy for these species is also confined to that used in previous treatments of tomatoes in the Galápagos; the synonymy of these cultivated species is extremely complex and has been made more so by the description of many garden-generated hybrids and a superfluity of *nomina nuda*. Complete synonymy of these species will be presented in Peralta *et al.* (in prep.)

Leaves of *Solanum* vary from simple to deeply to completely compoundly dissected. The leaves of tomatoes and their close relatives the potatoes have often been characterized as pinnate, but the presence of a minute wing of leaf tissue along the rachis connecting all dissections makes this distinction difficult to maintain in practice. We prefer to characterize the leaves of tomatoes as compoundly dissected following prevalent terminology in the current leaf development literature (Bharathan *et al.*, 2002; Gleissberg, 2002; Tsiantis *et al.*, 2002; but see Kessler *et al.*, 2001), although the species occurring in the Galápagos have nearly completely divided leaves that appear compoundly pinnate. We have followed common practice in using the term leaflet to mean a complete petiolate division of the blade. In the species descriptions leaf length excludes the petiole and interjected leaflets were defined as all leaflets along the rachis that are under half the length of the primary leaflets. Flower measurements were taken from live and dried plants, and seed weights represent dry seed weight. Terminology used in the descriptions follows that in Table 2. Detailed distributions for the introduced species are given for the Galápagos only, as both are widely cultivated all over the world.

Herbaria are cited using the acronyms in *Index herbariorum* (Holmgren *et al.*, 1990). Types seen are indicated by an exclamation mark (!), and we have seen all cited specimens and those in the exsiccatae unless otherwise indicated. Specimens examined are cited using the current accepted names for the islands of the Galápagos (Table 1).

Key to solanums in the Galápagos (the list of *Solanum* species currently occurring in the Galápagos Islands was obtained from the CDRS working database 2002)

1a. Plant with spines and stellate trichomes on at least some parts 2

1b. Plants without spines, if stellate trichomes present, then the inflorescence many-branched 4

2a. Leaves markedly bicoloured, white beneath; flowers white . . . . . **S. marginatum** L.f. (probably present, no herbarium specimen)

2b. Leaves not markedly bicoloured; flowers white or purple 3

3a. Leaves large and repand, densely pubescent and the trichomes flushed with purple; fruit orange with green flesh when ripe; flowers white . . . . . **S. quitoense** Lam. (escaped)

3b. Leaves not repand, the trichomes white or translucent; fruit purple or white when ripe, the flesh cream; flowers purple . . . . . **S. melongena** L. (cultivated)

4a. Leaves deeply pinnatifid and divided, with interjected leaflets 5

4b. Leaves simple, if pinnatifid, without interjected leaflets 6

5a. Flowers white or purple; plants with underground tubers; ripe fruits green . . . . . **S. tuberosum** L. (escaped)

5b. Flowers yellow; plants without underground tubers; fruit brightly coloured red, orange or yellow when ripe . . . . . **Solanum** section **Lycopersicon** (see key below)

6a. Shrubs or small trees; inflorescences usually branched; flowers greater than 1 cm in diameter 7

6b. Herbs; inflorescences usually simple; flowers smaller than 1 cm in diameter 8
7a. Leaves densely pubescent with stellate trichomes, the plant appearing woolly; leaf base acute; fruit *c.* 1 cm in diameter, globose, yellowish green when ripe ..... **S. erianthum** D. Don (naturalized)
7b. Leaves glabrous; leaf base cordate; fruit larger than 1 cm in diameter, ellipsoid, red, orangish red or pinkish when ripe ..... **S. betaceum** Cav. (cultivated)
8a. Leaves simple, the margins sinuate, entire or dentate; inflorescence umbelliform; plant glabrous or with simple non-glandular trichomes ..... **S. americanum** L.
8b. Leaves shallowly pinnatifid; inflorescence cymose; plant sticky with glandular trichomes ..... **S. edmonstonei** Hook.f. (endemic)

Keys to the tomatoes in the Galápagos

Artificial dichotomous key

1a. Leaflet margins lobed 2
1b. Leaflet margins more or less entire 5
2a. Tertiary lobing present, often tertiary leaflets with quaternary lobing; secondary leaflets per leaf more than five (usually more than ten); sepal length often exceeding fruit diameter when ripe; plants often densely hairy with glandular trichomes; plants often found on coastal lava ..... 2. **S. galapagense**
2b. Tertiary lobing absent; secondary leaflets, if present, fewer than 15 per leaf; sepal length not exceeding fruit diameter when ripe; plants found in a variety of habitats 3
3a. Ripe fruit yellow to deep orange, sometimes hairy; staminal column 4–7 mm long; bract-like leaves sometimes present in inflorescence; inflorescence sometimes branched; sepals usually appressed on to fruit; plants sometimes found on coastal lava ..... 1. **S. cheesmaniae**
3b. Ripe fruit bright red, glabrous; staminal column 6–9 mm long; bract-like leaves not present in inflorescence; inflorescence not branched; sepals on fruit reflexed; plants of disturbed areas, not from coastal lava beds 4
4a. Leaflet margins shallowly lobed mainly towards the base; foliage when crushed with citrus odour; fruit less than 20 mm in diameter when ripe; corolla deeply stellate, the lobes divided almost to the base; stems slender with occasional long trichomes up to 2.2 mm ..... 4. **S. pimpinellifolium**
4b. Leaflet margins deeply lobed along whole margin; foliage when crushed without citrus odour; ripe fruit over 20 mm in diameter; corolla shallowly stellate, the lobes divided 1/3 to 1/2 way to base; stems robust with occasional long trichomes up to 3 mm ..... 3. **S. lycopersicum**
5a. Plants found on coastal lava at 0–5 m elevation; leaves fleshy and sticky to touch ..... 1. **S. cheesmaniae**
5b. Plants found above 5 m elevation; leaves membranous or fleshy, not markedly sticky to the touch 6
6a. Ripe fruit yellow to deep orange, sometimes pubescent; sepals partially fused at base, free to only 2/3 of the way to the base, appressed on to fruit (not always apparent in herbarium specimens); seed weight 0.4–0.8 mg; staminal column 4–7 mm long; bract-like leaflets sometimes present in inflorescence ..... 1. **S. cheesmaniae**
6b. Ripe fruit bright red, glabrous; sepals stellate, free almost to the base, markedly reflexed along their entire length in fruit; seed weight greater than 0.8 mg; staminal column 6–8 mm long; bract-like leaflets not present in inflorescence ..... 4. **S. pimpinellifolium**

Synoptic key

Habitat and habit
- Plants found within 5 m of the high tide mark: che, gal
- Plants found in the highlands: che, pim, lyc, gal
- Stems with dense short pubescence: che, gal
- Stems with sparse elongate uniseriate, multicellular trichomes 2–3 mm: pim, lyc
- Stems more or less glabrous: che, pim
- Foliage with strong citrus odour: che, gal, pim

Leaves
- Leaflets margins entire: che, pim
- Leaves fleshy: che, gal
- Leaves sticky: che, gal
- Leaves membranous: che ('Academy Bay' morph), pim, lyc
- Leaves with tertiary lobes/leaflets: gal
- Leaves with 10 or more secondary lobes/leaflets: gal

Flowers
- Corolla lobes narrowly lanceolate, narrower than 1/3 the length of lobe from tip to flower centre, the corolla stellate: pim
- Corolla lobes deltate, wider than 1/3 the length of lobe from tip to flower centre, the corolla more pentagonal: che, gal, lyc

Fruit
- Fruit yellow when ripe: che
- Fruit orange when ripe: che, gal
- Fruit bright red when ripe: pim, lyc
- Sepal lobes strongly reflexed in fruit: pim, lyc
- Sepal lobes appressed on to fruit (not always apparent in herbarium specimens): che, gal
- Sepals in fruit longer than berry: gal

1. **Solanum cheesmaniae** (L. Riley) Fosberg, *Phytologia* **62**: 181 (1987). *Lycopersicon cheesmaniae* L. Riley (as *cheesmanii*), *Kew Bull.* **1925**: 227 (1925). Type: Ecuador. Galápagos. Santa Cruz: 'Indefatigable, among lava rock in grassy patches', 28 July 1924, *Cheesman in Riley 403* (K!-holotype).

Lycopersicon peruvianum (L.) Mill. var. *parviflorum* Hook.f., *Trans. Linn. Soc. London* **20**: 202 (1847) ('1851'). as '*L. peruanum*' Type: Ecuador. Galápagos. San Cristóbal: 'Chatham Island', end of September 1835, *Darwin s.n.* (K!-holotype).

Figs 4A–G; 6A, B.

Perennial herbs, undergoing secondary growth at the base; branches somewhat robust to slender and erect to vining,

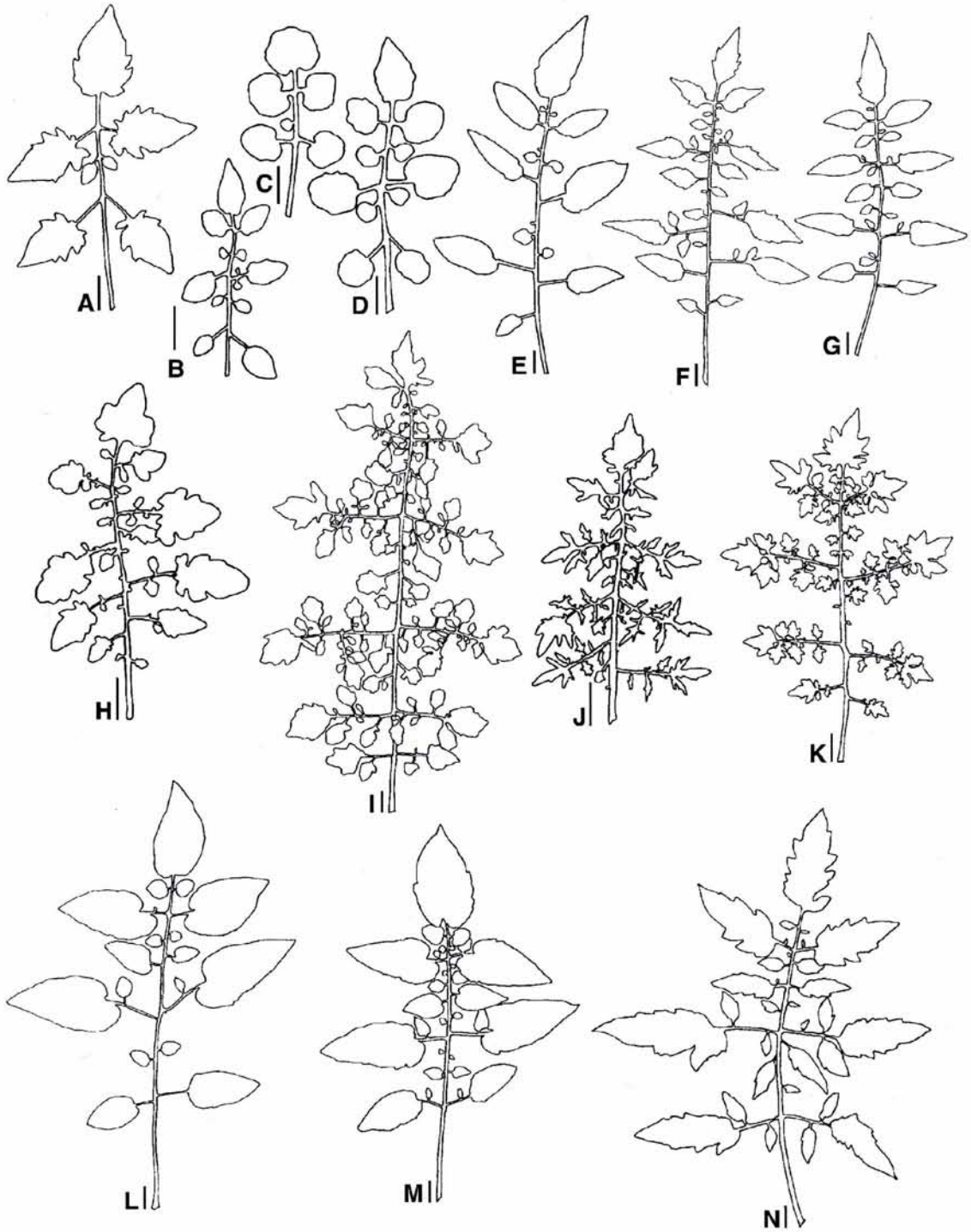


Figure 4 Leaf morphology variation in the Galápagos tomatoes. Scale bars all equal 1 cm. (h) – herbarium specimen, (g) – greenhouse grown plant. A–D. *Solanum cheesmaniae*, typical morph, A – North coast of Santa Cruz, *S. Darwin et al. 236.1* (g); B – North coast of Santa Cruz, *S. Darwin et al. 236* (h); C – North coast of Baltra, *S. Darwin et al. 202*, immature leaf (h); D – North coast of Baltra, *S. Darwin et al. 202*, mature leaf (h). E–G. *Solanum cheesmaniae*, 'Academy Bay' morph, E – El Lagoon del Manzanillo, *S. Darwin & Rosero 366* (h); F – Academy Bay, Santa Cruz, *Howell 9096* (h); G – Academy Bay, Santa Cruz, *Bentley 342* (h). H–K. *Solanum galapagense*, H – Bartolomé, *S. Darwin & Schultz 183.3* (g); I – El Lagoon del Manzanillo, *S. Darwin & Rosero 364* (h); J – Rabida, *Day 287* (g); K – Pinzón TGRC LA-0532 (g). L–M. *Solanum pimpinellifolium*, L – El Chato, Santa Cruz, *S. Darwin et al. 403* (h); M – Los Gemelos, Santa Cruz, *S. Darwin et al. 196* (h). N – *Solanum lycopersicum*, Villamil, Isabela, *S. Darwin 289* (h).

extending up to 2 m from centre. **Stem** erect initally, later procumbent or decumbent, variously pubescent, coastal populations more glandular; trichomes of several types, the longer *c.* 1 mm, simple, uniseriate, patent, amongst uniseriate, 1–2-celled slender trichomes and shorter glandular, simple, 1–2-cellular trichomes, the glands unicellular or multicellular, the plant with a strong citrus-like scent. **Sympodial units** trifoliate. **Internodes** 1.5–5(–8) cm. **Leaves** interrupted imparipinnate, 3.5–14 × 1.5–8.5 cm, sparsely pubescent to glabrescent ('Academy Bay') adaxially, densely pubescent with uniseriate uni- or multicellular downy trichomes abaxially, lime green to dark green; primary leaflets 2–3(–4) pairs, opposite, subopposite or alternate, 0.8–6 × 0.4–2 cm, ovate or orbicular, the base asymmetric, rounded to cordate, the margins entire to irregularly lobed; terminal primary leaflet usually larger than the laterals, about half as long as the leaf rachis; secondary leaflets present mainly basiscopically, 0–5(–8) per leaf; tertiary leaflets absent; interjected leaflets usually present, 4–8(–14), opposite, subopposite or alternate, sessile to short-petiolate; rachis 1.0–9.5 cm. **Petiole** 0.5–30(–35) cm, sparsely pubescent; pseudostipules absent. **Inflorescences** simple or sometimes 2–3-branched, to 7.5 cm, to 11-flowered, bract and bracteole-like leaflets sometimes present on the axis; peduncle 1–3.3 cm; rachis pubescent like the stems; pedicels 0.6–2 cm, articulate in the upper 1/3, occasionally without an articulation (from Academy Bay, see Rick, 1967). **Calyx** *c.* 0.6–1.2 cm in diameter, pubescent with long and short simple uniseriate trichomes; tube 0.5–1 mm; lobes 3–5 × *c.* 1 mm, linear, the apex acute. **Corolla** 1.8–2.8 cm in diameter, yellow; tube to 0.2 cm long; lobes 0.9–1.4 × 0.25–0.4 cm, narrowly deltate, reflexed at anthesis. **Staminal column** 4–7 mm, narrowly cone-shaped; filaments 1–2(–2.5) mm; anthers 3–5 mm, the sterile tip 1–3 mm. **Ovary** conical, minutely glandular villous; style 3–6(–8) mm, usually included in the staminal column, but exserted to 1(–2) mm in some specimens; stigma minute. **Fruits** 0.6–1.4(–2.5) cm in diameter, globose and 2-locular, glabrescent and becoming yellow or orange at maturity; calyx lobes in fruit accrescent, to 0.45–1.3 × 0.5–0.3 cm, tightly appressed or spreading. **Seeds** (5–)20(–45) per fruit, *c.* 1.5–2.2 × 1 mm, with a pronounced beak; testa appearing hairy over entire surface with the elongate lateral cell walls; dry seed weight *c.* 0.6 mg.

DISTRIBUTION. Endemic to the Galápagos Islands, found both on coastal lava 1 m above sea level within the range of sea spray and at higher altitudes (Fig. 1).

COMMON NAMES. Tomatillo, Galápagos tomato.

USES. Putatively edible, although rather sharp to taste (SD, pers. obs.). Germplasm has been used to enhance cultivated tomatoes for the joint-less pedicel character found in some plants in Academy Bay, Santa Cruz (Rick, 1967).

REPRESENTATIVE SPECIMENS EXAMINED. (* indicates specimens of the 'Academy Bay' morph.)

ECUADOR. Galápagos. Baltra: N. coast, coastal lava, 3 m, 00°24′86″S, 90°17′23″W, 3 July 2000, *Darwin, S. et al. 203* (BM, CDS); N. coast, coastal lava, 3 m, 00°24′86″S, 90°17′23″W, 3 July 2000, *Darwin, S. et al. 205* (BM, CDS); N. coast, coastal lava, 3 m, 00°24′86″S, 90°17′23″W, 3 July 2000, *Darwin, S. et al. 209* (QCA, QCNE). **Española**: sin. loc., 8–20 July 1983, *Touc s.n.* (CDS). **Fernandina**: alluvial fan near W. coast, periodically flooded *Bursera* forest, 25 m, 19 September 1974, *Adsersen & Adsersen 903** (C, CDS); SE slope, approx. 2.5–3.5 km below the rim of the caldera, 740 m, 22 January 1972, *Hamann & Hamann 213** (C); en la cumbre al oeste de la caldera, en pequeñas manchas densas dentro del 'bosque' de *Scalesia*, 1300 m, 00°20′S, 91°31′W, 7 December 1984, *Huttel 495** (CDS, QCA). **Isabela**: Volcán Darwin, Islote Crater Beagle 2, entre las rocas, 11 June 1994, *Aldaz 350* (CDS); sin. loc., 1853, *Andersson s.n.* (S); Volcán Alcedo, from sea level nearly to the top, 18 August 1963, *Castro s.n.* (CDS); just outside Villamil, by El Lagoon del Manzanillo, growing next to gravel pit formed due to the extraction of gravel for the airport built in 1996, 16 m, 00°55′85″S, 90°58′68″W, 25 July 2000, *Darwin, S. & Rosero 365** (BM, CDS, QCNE); just outside Villamil, by El Lagoon del Manzanillo, growing next to gravel pit formed due to the extraction of gravel for the airport built in 1996, 16 m, 00°55′85″S, 90°58′68″W, 25 July 2000, *Darwin, S. & Rosero 366** (BM, CDS, QCNE); Caleta Black, sea shore, 0–10 m, 3 June 1959, *Eliasson & Eliasson 2207* (S); Volcán Alcedo, W. slope of caldera, 500 m, 14 July 1972, *Hamann & Hamann 1801** (C); *Harling 5288* (S); Volcán Wolf, E. side, 1170 m, 21 May 1967, Iguana Cove, 21 May 1932, *Howell 9427** (CAS); 5 miles N. of Webb Cove, 22 May 1932, *Howell 9447* (CAS); 3 miles S. of the equator, E. side of island, 30 May 1932, *Howell 9617* (CAS); Volcán Wolf, 11 April 1986, *Lawesson 3017** (CDS); Volcán Darwin, 13 April 1986, *Lawesson 3080** (CDS); Iguana Cove, abundant on side of cliff above the cove, 20 March 1905–1906, *Stewart 3379** (CAS, GH, US). **Pinzón**: NW slope of island, a square 5-ha area with its SE corner in MacFarland's (Director CDRS) old camp, crossed by trails to crater and to W. slope tortoise-nesting zone (area includes 'union de dos caminos'), rocky, dry thorn-scrub, *Prosopis juliflora, Croton scouleri*, 18 April 1975, *Clark & Clark 344* (WIS). **San Cristóbal**: sin. loc., end September 1835, *Darwin, C. s.n.* (K); sin. loc., end September 1835, *Darwin, C. s.n.** (CGE); sin. loc., end September 1835, *Darwin, C. s.n.* (CGE); champ de laves récentes au NE de Cerro Brujo, préférence pour laves acoriacées, 75 m, 6 December 1988, *Huttel 1597* (CDS, QCA); Sappho Cove, occasional on recent lava, 18 February 1905–1906, *Stewart 3374* (CAS, GH). **Santa Cruz**: Charles Darwin Research Station, along path running between town station road and tortoise-rearing house, in sunny area, 2 May 1983, *Bentley 342** (CDS, K, QCA, US); Academy Bay, collected on edge of 'barranco' at Puerto Ayora, 20 m, 13 April 1953, *Bowman 119** (CAS, UC); 1.5 miles N. Academy Bay, 20 m, 20 April 1953, *Bowman 120** (CAS, UC); 1 km NW of Cerro Colorado, coastal lava, 20 m, 00°33′95″S, 90°10′54″W, 5 July 2000, *Darwin, S. et al. 214* (BM, CDS); *c.* 5 km NW of Cerro Colorado, coastal lava, < 15 m, 00°32′63″S, 90°12′50″W, 5 July 2000, *Darwin, S. et al. 236* (BM); *c.* 5 km NW of Cerro Colorado, coastal lava, < 15 m, 00°32′63″S, 90°12′50″W, 5 July 2000, *Darwin, S. et al. 239* (BM); Punta Carrion, coastal lava, 4 m, 00°28′91″S, 90°15′06″W, 5 July 2000, *Darwin, S. et al. 272* (BM, CDS); Academy Bay, 0–10 m, 4 October 1966,

*Eliasson & Eliasson 201** (MO, S); N. slope, *c.* 100 m from road between Santa Rosa and canal, dry seasonal deciduous steppe forest, 130 m, 17 March 1981, *Hamann & Seberg 1771* (C); Academy Bay, 4 May 1932, *Howell 9096** (CAS); Academy Bay, semi-open habitat, lava (Halboffen, lavagelaende), 10 m, 25 June 1932, *Schimpff 12** (BM, CAS, G, M, MO, NY, S, U, Z); Punta Bowditch-Costa, en las pendientes de un pequeño crater entre la costa y el Cerro Montura, without date, *Huttel 2735* (CDS); N. side, common among rocks, 75 m, 24 November 1905–1906, *Stewart 3376* (CAS, GH). **Santa Fé**: stony barranca, 100 m, 16–17 June 1959, *Harling 5371* (S); rocky shore, 15 m, 16–17 June 1959, *Harling 5476* (S); W. part of island, highest plateau, 280 m, 16 February 1972, *Hamann & Hamann 444* (C); sin. loc., 14 September 1973, *de Vries 1227* (CDS).

In the morphological analyses, plants of *Solanum cheesmaniae* form a cohesive group despite considerable variation, and we identify two extreme morphs showing differences in leaflet shape, margin, leaf division and pubescence (Fig. 4A–G). The type specimen represents the 'typical' morph, and has very small leaves and leaflets, with entire to regularly dentate margins (Fig. 4A–D) and dense pubescence in all parts of the plant with short glandular trichomes on the adaxial surface of the leaflet. These characters are consistently present on specimens collected from the north coast of Santa Cruz and Baltra, San Cristóbal, Santa Fé, Pinzón and coastal Isabela. The other extreme morph in *S. cheesmaniae*, which we here call the 'Academy Bay' morph, has leaves up to three times the size of the 'typical' morph, irregularly dentate leaflet margins (Fig. 4F–G), and the plants are altogether less pubescent; the lack of trichomes is especially notable on both leaflet faces. The 'Academy Bay' morph has been collected from near areas of human habitation on southern Santa Cruz (Academy Bay = Puerto Ayora). Other specimens, that we here consider to fall within the 'Academy Bay' morph, show intermediate leaf morphology compared with the two extremes (Fig. 4E). These plants have a velvety pubescence due to short trichomes of similar length; this pubescence is more apparent on the abaxial face of the leaflets. These intermediates have been collected from Isabela, Fernandina and Santa Cruz, and also possibly occur on San Cristóbal and Española. Specimens of the 'Academy Bay' morph have been collected mostly from the southern sides of the islands or at high altitudes (the areas where there is maximum precipitation). Many of these specimens were collected during El Niño years (see Quinn & Neal, 1992 for a list of El Niño dates).

Hooker (1847) recognized three different species of tomatoes from the Galápagos, all based on the specimens collected by Darwin (Fig. 2). We found that one of these specimens documented as having been collected from San Cristóbal, and identify by Hooker as '*L. pimpinellifolium*', belongs to the 'Academy Bay' morph (Fig. 2). Some of the specimens cited by Müller (1940), as '*Lycopersicon pimpinellifolium*' are also identified here as *S. cheesmaniae* 'Academy Bay' morph. Rick (1956, 1963) referred to three different tomatoes in Galápagos including a Galápagos '*L. pimpinellifolium* type' (TGRC accession number LA166), which had orange fruit but flowers with corolla divided two thirds the way to the base (for illustration see Rick, 1956). In his later work, Rick (1971) re-classified the Galápagos accessions of '*L. pimpinellifolium*' with orange fruits under '*L. cheesmanii*'. He considered the red-fruited tomatoes in Galápagos to be introduced species (Rick in litt., 1998). The leaf morphology of some specimens of *S. cheemaniae* 'Academy Bay' morph from Isabela is similar to individuals of *S. pimpinellifolium* found in Galápagos and on mainland South America (coastal Perú and Ecuador).

The morphological variation in this species is indeed complex. Rick (1963) also recognized this and pointed out that *S. cheesmaniae* from the northern side of Santa Cruz was intermediate in terms of pubescence density between *S. galapagense* and what we here define as the 'Academy Bay' morph of *S. cheesmaniae*. He also observed that 'typical' *S. cheesmaniae* shared morphological similarities with the 'Academy Bay' morph (Fig. 4E–G) but resembled *S. galapagense* (Fig. 5) with respect to its shorter internodes. Rick (1963) felt that the typical *S. cheesmaniae* had leaves that were less divided than two other forms and with orbicular lateral segments (Fig. 4A–D). Our observations are consistent with these morphological differences noted by Rick (1963).

A comparison between herbarium specimens and greenhouse-grown progeny collected from the same plants showed that the greenhouse-grown plants had larger leaf dimensions than their field-collected parents (Fig. 4A, B). Size difference, however, did not wholly account for the differences between the typical *S. cheesmaniae* and the 'Academy Bay' morphs (Fig. 4A–G). Further investigations are being undertaken to resolve the relationships and taxonomic status of the 'Academy Bay' morph and to establish the reasons for these different morphologies found within *S. cheesmaniae*. Variation in *S. cheesmaniae* is potentially due to a variety factors. These include: (1) plants here recognized as the 'Academy Bay' morph could be an ecotype of *S. cheesmaniae*; (2) plants are potentially of hybrid origin involving *S. pimpinellifolium*; or (3) plants could be morphologically aberrant due to increased soil humidity found in the southern parts of the islands and during El Niño years.

The spelling of the specific epithet has been corrected from *cheesmanii* to *cheesmaniae*, as Evelyn Cheesman, the collector of the type and in whose honour the species was named, was a woman (see Fosberg, 1987; Spooner *et al.*, 1993; Greuter *et al.*, 2000). Although Fosberg (1987) pointed this out, usage has not changed in the plant breeding literature, but floristic studies have consistently used the correct spelling (Jørgensen & León-Yánez, 1999).

The publication date of Hooker's *Enumeration of Galapagos Plants*, in which he described both *Lycopersicon peruanum* var. *parviflorum* (= *Solanum cheesmaniae*) and *Lycopersicon esculentum* var. *minor* (= *Solanum galapagense*) is given on the frontispiece of Volume 20 of the *Transactions of the Linnean Society of London* as 1851, but Part II, in which the paper appeared (read on 4 May, 6 May and 16 December 1845) was available as a separate on 11 December 1847 (Raphael, 1970). Thus the correct date of publication of the names published therein is 1847, not 1851 as it appears in most bound library copies of the *Transactions*. This accounts for considerable confusion over the dates of publication of these

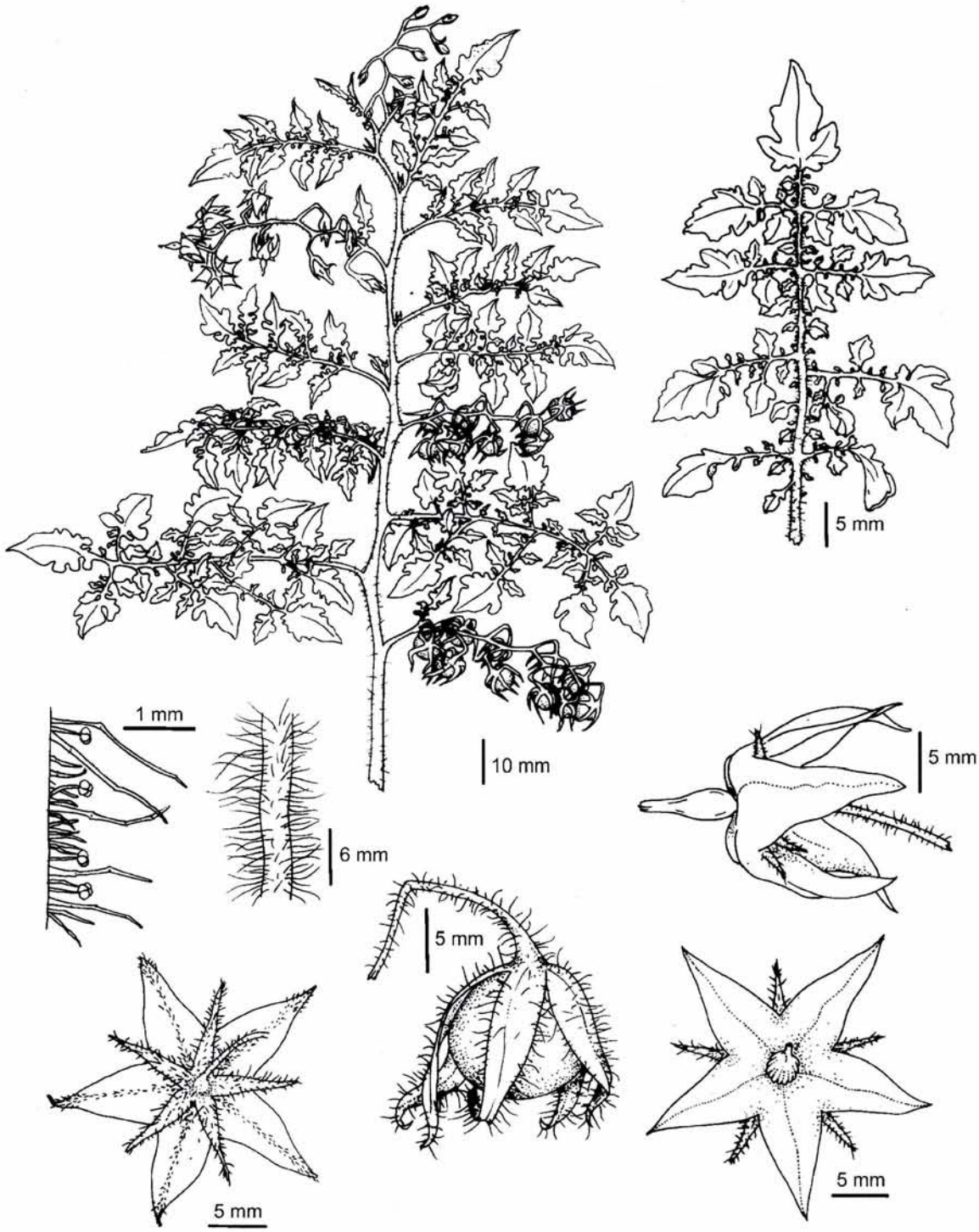


Figure 5 *Solanum galapagense* S. Darwin & Peralta (based on *S. Darwin & Schultz 190*, BM).

epithets and is further complicated by the publication, in 1846, of a summary of the reading of Hooker's enumeration. This publication, issued in the *Proceedings of the Linnean Society of London*, included some new generic descriptions (Hooker, 1846). No tomato taxa were mentioned in this 1846 work.

2. **Solanum galapagense** S. Darwin & Peralta, **sp. nov.** Type: Ecuador. Galápagos: Isla Bartolomé, E. of the saddle beach, *c.* 6 m, 0°17′01″S, 90°33′30″W, 28 June 2000, *S. Darwin & Schultz 184* (CDS!-holotype; BM!-isotype).

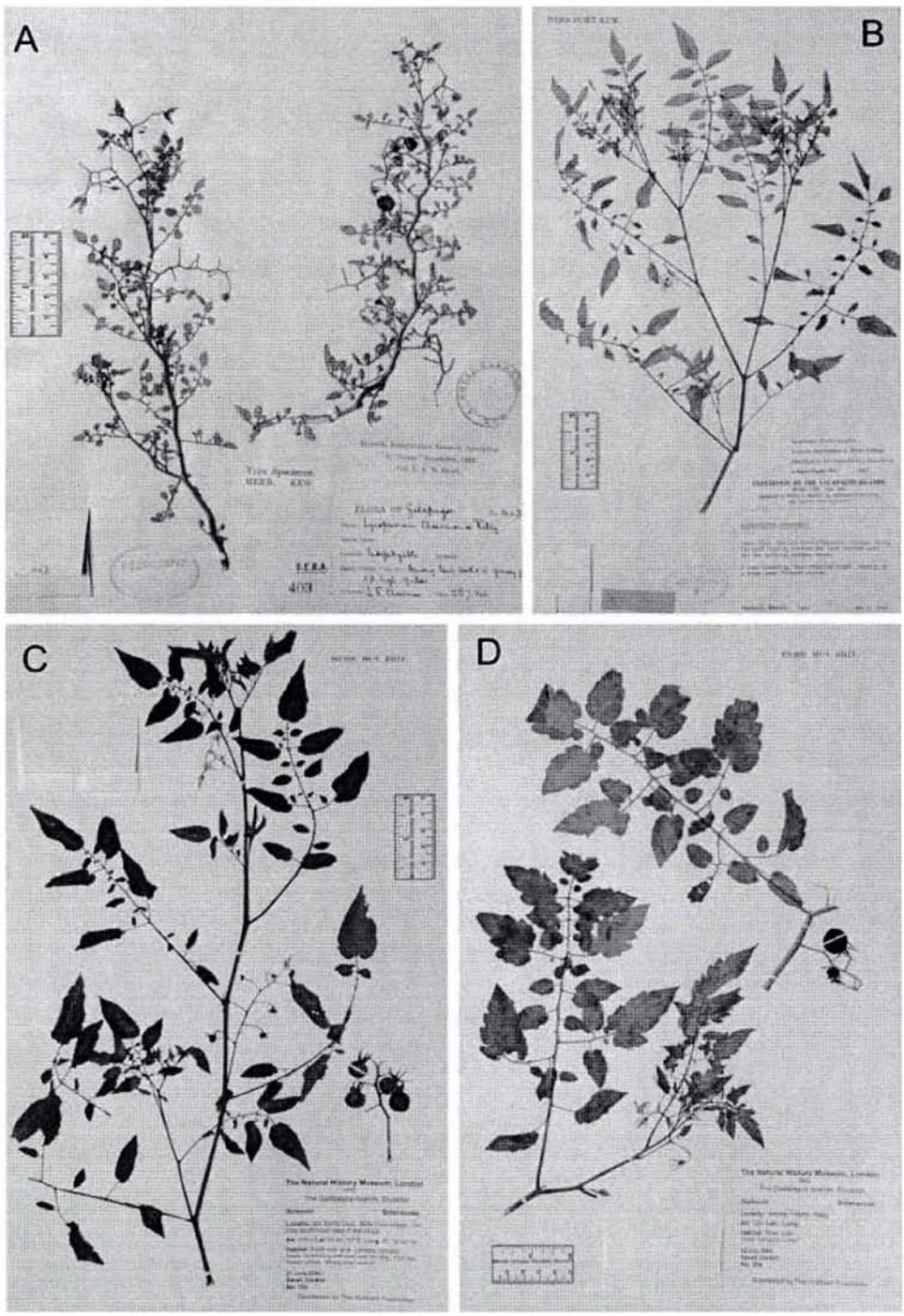


Figure 6 *Solanum* section *Lycopersicon* in the Galápagos. A. *Solanum cheesmaniae*, typical morph, B. *Solanum cheesmaniae*, 'Academy Bay' morph, C. *Solanum pimpinellifolium*, D. *Solanum lycopersicum*.

Lycopersicon esculentum Mill. var. *minor* Hook.f., *Trans. Linn. Soc. London* **20**: 202 (1847) ('1851'). *Lycopersicon cheesmaniae* L. Riley forma *minor* (Hook.f.) C.H. Müll, *U.S. Dept. Agric. Misc. Publ.* **382**: 21 (1940). *Lycopersicon cheesmaniae* L. Riley var. *minor* (Hook.f.) D.M. Porter, *Madroño* **25**: 58 (1978). Type: Ecuador. Galápagos. Santiago: 'James Island', October 1835, *Darwin s.n.* (CGE!-holotype).

Figs 4H–K, 5.

Species *S. cheesmaniae* baccibus aurantiacis similis, foliis valde divisis foliolis tri-dissectis, sepalis in fructo bacca longiori differt.

Perennial herbs, undergoing secondary growth at the base; branches somewhat robust to slender, erect to vining, extending up to 2 m from centre. **Stem** erect initially, later procumbent

or decumbent, densely pubescent; trichomes of several lengths, the longest 0.5–2 × *c.* 0.1 mm, simple, uniseriate, some gland-tipped, the longer ones with minute single-celled glands, among dense uniseriate 1–2-celled trichomes, some gland-tipped, the glands unicellular and minute or multicellular, the plant with a strong citrus scent, smaller trichomes unicellular, uniseriate and usually gland-tipped. **Sympodial units** trifoliate. **Internodes** 1.5–3.5(–6) cm. **Leaves** interrupted imparipinnate, 5–25 × 2–17 cm, densely pubescent with uniseriate glandular trichomes *c.* 0.5 mm and shorter unicellular trichomes on both surfaces, denser abaxially, lime green; primary leaflets 2–4 pairs, subopposite or alternate, 2–7 × 1–4.5 cm, ovate or obovate, the base asymmetric, cuneate to cordate, the margins deeply lobed, forming secondary, tertiary and occasionally quaternary leaflets of varying sizes; terminal primary leaflet scarcely larger than the laterals; secondary leaflets present, always more than (6–)10–30 per leaf; tertiary leaflets usually present; interjected leaflets usually present, (3–)5–22(–30), subopposite or alternate, sessile to short-petiolate; rachis 12–18.5 cm. **Petiole** 0.6–4 cm, sparsely pubescent; pseudostipules absent. **Inflorescences** simple or occasionally 2–3-branched, to 10 cm, to 12-flowered, bract and bracteole-like leaflets occasionally present in some populations; peduncle 1–3.5 cm; rachis pubescent like the stems; pedicels 0.5–1.8 cm, articulate just below the middle. **Calyx** 0.6–1.2 cm in diameter, pubescent with long and short simple uniseriate trichomes; tube 0.5–1 mm; lobes 3–6 mm, linear, the apex acute. **Corolla** 1.6–3.2 cm in diameter, yellow, occasionally somewhat bilaterally symmetric due to fusion of adjacent lobes; tube 0.5–0.7 cm long; lobes 0.7–1.3 × 0.3–0.7 cm, deltate to narrowly deltate, reflexed at anthesis. **Staminal column** 3–7 mm, narrowly cone-shaped; filaments 1–2.75 mm; anthers 3–4.5 mm, the sterile tip 1–2(–4) mm. **Ovary** conical, minutely glandular-villous; style 4–8 mm, usually included in the staminal column, rarely exserted to less than 0.5 mm; stigma minute. **Fruits** 0.6–1.1 cm in diameter, globose and 2-locular, glabrescent to densely pubescent with simple uniseriate patent trichomes becoming pale to deep orange at maturity; calyx lobes in fruit accrescent, *c.* 1.4 × 0.1–0.3 cm, longer than fruit, basal half of sepals tightly appressed to berry base, the pedicels in fruit curving towards the axis. **Seeds** (5–)*c.* 30(–50) per fruit, *c.* 2 × 1 mm, usually beaked; testa appearing hairy over entire surface with the elongate lateral cell walls; dry seed weight *c.* 0.5 mg.

DISTRIBUTION. Endemic to the Galápagos Islands, particularly the western and southern islands, mostly occurring on coastal lava (see cover photograph) to within 1 m of high tide mark within range of sea spray (strongly salt tolerant) but also occasionally inland, for example on Isabela and Fernandina (Fig. 1).

COMMON NAMES. Tomatillo, Galápagos tomato.

USES. Putatively edible, although rather sharp to taste (SD, pers. obs.). Germplasm has been used to enhance salt tolerance in cultivated tomatoes (Tal & Shannon, 1983) and high fruit content of soluble solids (Garvey & Hewitt, 1991; Triano & St Clair, 1995).

REPRESENTATIVE SPECIMENS EXAMINED

ECUADOR. Galápagos. Sin. loc., 1827, *Scouler s.n.* (E). **Bartolomé**: to right of summit landing site, coastal lava, 2 m, 00°14′72″S, 90°33′12″W, 28 June 2000, *Darwin, S. & Schultz 181* (CDS, QCNE); coastal lava, 2 m, 00°14′72″S, 90°33′12″W, 28 June 2000, *Darwin, S. & Schultz 182* (BM, CDS); E. of the saddle beach, coastal lava, 6 m, 00°17′01″S, 90°33′30″W, 28 June 2000, *Darwin, S. & Schultz 189* (CDS); E. of the saddle beach, coastal lava, 6 m, 00°17′01″S, 90°33′30″W, 28 June 2000, *Darwin, S. & Schultz 190* (BM); rocas o lava, muy comun en las zona arida de la isla, 50 m, 00°16′41.6″S, 90°32′53.6″W, 19 July 1997, *Jaramillo 1052* (CDS); sin. loc., *Fagerlind & Wibon 3464* (S); on barren lava and in cinder patches among lava blocks and flows, 10–30 m, 3 February 1967, *Wiggins & Porter 296* (CAS, GH, SGO); barren lava along E. side of lava ridge *c.* 2 km from W. end of Isla San Bartolomé, 50 m, 3 February 1967, *Wiggins & Porter 314* (CAS, USN). **Corona del Diablo**: sin. loc., 10 m, 23 November 1966, *Eliasson & Eliasson 656* (S). **Darwin**: sin. loc., October 1983, *Touc s.n.* (CDS). **Española**: sin. loc., May 1899, *Snodgrass & Heller 741* (DS, GH); sin. loc., 8–26 July 1983, *Touc s.n.* (CDS). **Fernandina**: NW of rim, on 1968 ash deposit, 1300 m, 1974, *Adsersen & Adsersen 921* (C, CDS); green strip on SW slope, large clumps growing at edge of *Scalesia* zone on deep ash sand near crater rim, 1460 m, 4 February 1964, *Cavagnero 25* (MO); SW corner of island, Cabo Hammond, 27 April, *Reeder s.n.* (WIS); NW slope, 700–1200 m, September 1972, *Schmidt & Schmidt 2528* (C). **Gardner (nr. Española)**: sin. loc., 3 October 1905–1906, *Stewart 3373* (CAS); **Gardner (nr. Floreana)**: sin. loc., 16–19 August 1983, *Touc s.n.* (CDS). **Isabela**: fumarole on E. saddle, lava, 380 m, 12 November 1974, *Adsersen & Adsersen 1165* (C); El Lagoon del Manzanillo, growing next to gravel pit formed due to extraction of gravel for airport built in 1996, 16 m, 00°55′85″S, 90°58′68″W, 21 July 2000, *Darwin, S. et al. 291* (BM, CDS, QCA, QCNE); El Lagoon del Manzanillo, growing next to gravel pit formed due to extraction of gravel for airport built in 1996, 16 m, 00°55′85″S, 90°58′68″W, 21 July 2000, *Darwin, S. et al. 294* (CDS); just outside Villamil, by El Lagoon del Manzanillo, growing next to gravel pit formed due to extraction of gravel for airport built in 1996, 16 m, 00°55′85″S, 90°58′68″W, 25 July 2000, *Darwin, S. & Rosero 364* (BM, CDS, QCA, QCNE); Volcán Darwin, beach N. of Tagus Mountain, coastal, 0–5 m, 12 July 1972, *Hamman & Hamman 1729* (C); Punta Albemarle, 29 June 1961, *Lévêque 163* (MO); Sierra Negra, 10 km N. of Villamil, 75–80 m, 1 October 1972, *Hamann & Hamann 2483* (C); Tagus Cove, 120 m, 25 June 1963, *Snow s.n.* (CDS). **Pinta**: sin. loc., 460 m, 21 May 1964, *Castro s.n.* (CDS); sin. loc., 460 m, 21 May 1964, *Snow 591* (K); sin. loc., 200–500 m, 19 September 1905, *Stewart 3370* (CAS); nr fumerole, 12 October 1973, *de Vries s.n.* (CDS); SE – slope, 300 m, June 1975, *van der Werff 2129* (U); **Pinzón**: growing between rocks, 10 m, 7 February 1958, *Castro s.n.* (CAS). **Rábida**: among lava boulders on upper slopes of main volcanic peak, 22 March 1967, *DeRoy & DeRoy 11* (DS); sin. loc.,

6 June 1932, *Howell 9753* (CAS); barranca, N. slope, 300 m, 30 September 1975, *Reeder & Chapy s.n.* (WIS). **Santiago**: Caleta Bucanero, steep cleft, 10 m, 1 June 1977, *Adsersen & Adsersen 1771* (C, CDS); Sullivan Bay, 13 June 1932, *Howell 10012* (B, CAS, K); sin. loc., 1853, *Andersson s.n.* (BR); Crab Point (S. of James Bay–W. side of Island), coastal, in place inaccessable to goats, 16–20 August 1957, *Castro s.n.*(CAS); sin. loc., beginning October 1835, *Darwin, C. s.n.* (CGE); sin. loc., 5 June 1932, *Howell 9701* (CAS); sin. loc., June 1899, *Snodgrass & Heller 399* (GH); James Bay, 700–1600 m, 19 September 1905–1906, *Stewart 3369* (CAS); James Bay, 300 m, 6 August 1905–1906, *Stewart 3378* (BM, CAS, MO, NY, USN). **Sombrero Chino**: NE side of the islet, lava, 20 m, 00°22′15″S, 90°34′93″W, 28 June 2000, *Darwin, S. & Schultz 138* (QCA, QCNE); NE side of islet, lava, 20 m, 00°22′15″S, 90°34′93″W, 28 June 2000, *Darwin, S. & Schultz 139* (BM, CDS); NE side of islet, lava, 20 m, 00°22′15″S, 90°34′93″W, 28 June 2000, *Darwin, S. & Schultz 145* (QCNE); NE side of islet, lava, 20 m, 00°22′15″S, 90°34′93″W, 28 June 2000, *Darwin, S. & Schultz 149* (CDS); NE side of islet, lava, 20 m, 00°22′15″S, 90°34′93″W, 28 June 2000, *Darwin, S. & Schultz 157* (BM); sin. loc., 21 December 1993, *Snell 109* (CDS).

Solanum galapagense can be clearly differentiated from the other three taxa on leaf morphology alone. Other discriminating characters included appressed sepals that exceed the ripe fruit diameter, the presence of bract-like leaflets on the inflorescence and the presence of branched inflorescences. These morphological characters were found at a lower frequency in *S. cheesmaniae*, only rarely in *S. lycopersicum* and *S. pimpinellifolium*. Note that the presence of appressed sepals is not always apparent in herbarium specimens as sepals apparently curl upwards as they dry and can become reflexed.

Throughout the numerous different Galápagos tomato classifications there has been little doubt that *S. galapagense* is distinct; indeed, this is the only taxon that remains consistently separated throughout all the different treatments of tomatoes in the Galápagos (see Fig. 2). The morphological analysis indicates that *S. galapagense* is more distinct from the other three taxa than *S. pimpinellifolium* and *S. lycopersicum* are from each other.

Orange fruit colour is only found in *Solanum cheesmaniae* and *S. galapagense*. This character is derived in these two species (Peralta & Spooner, 2001) and morphologically separates them from *S. pimpinellifolium* and *S. lycopersicum* which in the Galápagos have unequivocally bright red fruit. Fruit colour was described by Rick (1971) as a 'dependable key character' with which to differentiate the Galápagos tomatoes from all others.

3. **Solanum lycopersicum** L., *Sp. Pl.* 185 (1753). *Lycopersicon esculentum* Mill., *Gard. Dict.* ed. 8, *Lycopersicon* No. 2 (1768). Type: Cultivated in Uppsala, *Anon.* (LINN 248.16!-lectotype, designated by Knapp & Jarvis, 1990 [BH neg. 6803: BH!, GH!, UC!, WIS!]).

Figs 4N, 6D.

Annual or biennial herbs, undergoing secondary growth at the base; branches relatively robust and vining, extending up to 1 m from centre. **Stem** erect initally, later procumbent or decumbent, pubescent and usually villous towards the apex; trichomes of two types, numerous simple unicellular trichomes and sparse simple, uniseriate trichomes up to 3 mm and composed of up to 10 cells, these usually denser at the nodes, the plant with a strong tomato scent. **Sympodial units** trifoliate. **Internodes** 1–4 cm, but very few specimens available. **Leaves** interrupted imparipinnate, 20–30 × 10–23 cm, sparsely pubescent like the stems on both surfaces or glabrescent, dark green; primary leaflets 2–3 pairs, opposite, subopposite or alternate, 3.5–8.5 × 1.3–3 cm, ovate or elliptic, the base asymmetric, truncate to cordate, the margins dentate or crenate mainly near the base, rarely deeply dentate or lobulate; terminal primary leaflet usually larger than the laterals; secondary leaflets fewer than 15 to absent, present mainly basiscopically; interjected leaflets usually present, 5–12 per leaf, subopposite or alternate, short-petiolate; rachis 1.9–14.5 cm. **Petiole** 1.2–4.2 cm, sparsely pubescent; pseudostipules absent. **Inflorescences** usually simple, rarely with 2 branches, to 5 cm, 5–8-flowered, shorter than the stems and growing leaves; peduncle 1–3.5 cm; rachis pubescent like the stems; pedicels 0.6–1.8 cm, articulate just above or just below the middle. **Calyx** to 1.8 cm in diameter, pubescent with long and short simple uniseriate trichomes; tube very short, less than 1 mm; lobes to 5–9 × 1 mm, linear, the apex acute. **Corolla** 2–3 cm in diameter, bright yellow; tube to 0.6 cm long; lobes 0.8–1.3 × 0.35–0.5 cm, narrowly lanceolate, spreading to somewhat reflexed at anthesis. **Staminal column** 6.5–8.5 mm, narrowly cone-shaped; filaments 1–3 mm; anthers 4–6.5 mm, the sterile tip less than half the total column length. **Ovary** conical, minutely glandular-villous; style 6.5–10 mm, usually included in the staminal column, but exserted in facultatively allogamous populations; stigma minute. **Fruits** (2–)2.2–4(–10 in some cultivars not known from Galápagos) cm in diameter, usually globose and 2-locular, but often of varying shape and multilocular, glabrescent and becoming red at maturity; calyx lobes in fruit accrescent, 0.75–1.6 cm, somewhat reflexed, never exceeding the length of the fruit. **Seeds** 25–85 per fruit, *c.* 3.5 × 2.5 mm, beaked; testa appearing hairy over entire surface with the elongate lateral cell walls; dry seed weight *c.* 1.5 mg.

DISTRIBUTION. Native distribution of the cultivated tomato is not known; in the Galápagos it is often found in rubbish dumps or near human habitation on Santa Cruz, Isabela and San Cristóbal. Probably introduced before 1932.

COMMON NAMES. Tomate, garden tomato, cherry tomato, cultivated tomato.

USES. Edible; widely used as a vegetable throughout the world.

SPECIMENS EXAMINED.

ECUADOR. Galápagos. Isabela: Villamil, roadside, <10 m, 21 July 2000, *Darwin, S. 289* (BM); Villamil village, roadside, 10 m, 22 July 2000, *Darwin, S. 304* (CDS); San Tomas near Villamil, waste land, 330 m, 00°51′25″S, 90°01′54″W, 22 July 2000, *Darwin, S. et al. 302* (BM, CDS); Villamil, active rubbish dump, 133 m, 00°52′66″S, 90°00′42″W, 25 July 2000, *Darwin, S. & Rosero 354* (CDS); road from Villamil to San

Tomas, active rubbish dump, 133 m, 00°52′66″S, 90°00′42″W, 25 July 2000, *Darwin, S. & Rosero 358* (BM, CDS, QCA); road from Villamil to San Tomas, active rubbish dump, 133 m, 00°52′66″S, 90°00′42″W, 25 July 2000, *Darwin, S. & Rosero 360* (BM, CDS); Villamil, by ECCD office, roadside, 10 m, 25 July 2000, *Darwin, S. & Rosero 376* (CDS). **San Cristóbal**: Wreck Bay, 17 April 1932, *Howell 8573* (B, CAS). **Santa Cruz**: between Puerto Ayora and Bella Vista, trackside, 1953, *TGRC accession LA0292* (TGRC seed bank).

The cultivated tomato *S. lycopersicum* is grown wherever people establish towns and villages, and it is a quick-growing adventive weed in many parts of the world. The date of its introduction to the Galápagos is uncertain, as settlement on the islands was explosive in the last century. Rick (1963) mentions '*Lycopersicon esculentum* var. *cerasiforme*' (TGRC accession LA292 (SCD 067)) collected in 1952, as occurring as a garden escape between Puerto Ayora (Academy Bay) and Bella Vista on Santa Cruz. The earliest herbarium specimen for *S. lycopersicum* is *Howell 8573*, collected in 1932 from San Cristóbal.

4. **Solanum pimpinellifolium** L., *Cent.* 8 (1755). *Lycopersicon pimpinellifolium* (L.) Mill., *Gard. Dict.* ed. 8, *Lycopersicon* No. 4 (1768). Type: Cultivated in Uppsala, *Anon.* (LINN 248.15!-lectotype, designated by Knapp & Jarvis, 1990 [BH neg. 6802: GH!, UC!, WIS!]).

Figs 4L–M, 6C.

Annual or biennial herbs, undergoing secondary growth at the base; branches extremely slender and vining, extending up to 3 m from centre. **Stem** erect initally, later procumbent or decumbent, sparsely pubescent or nearly glabrous; trichomes of two types, the longer sparse to extremely sparse and occasionally absent, to 2.2 mm, simple, uniseriate, to 6-celled, amongst sparse shorter, unicellular non-glandular and glandular trichomes, the glands usually multicellular, the plant with a citrus scent. **Sympodial units** trifoliate. **Internodes** 1.5–6.5(–7.5) cm. **Leaves** interrupted imparipinnate, 5–20 × 2.5–15 cm, sparsely pubescent like the stems on both surfaces, less pubescent adaxially, dark green, often with purplish cast abaxially; primary leaflets 2–3 pairs, opposite, subopposite or alternate, 3–7 × 1–4 cm, ovate, the base asymmetric, cuneate to cordate, the margins entire or irregularly lobed mainly near the base; terminal primary leaflet usually larger than the laterals, approximately equal in length to the leaf axis; secondary leaflets fewer than 6, often absent; tertiary leaflets absent; interjected leaflets usually present, 2–12(–15), subopposite or alternate, short-petiolate; rachis 2.0–15 cm. **Petiole** 0.4–3.0(–5.5) cm, glabrous or with a few uniseriate trichomes; pseudostipules absent. **Inflorescences** simple, very rarely once-branched, elongate, to 9 cm, 5–6-flowered, shorter than the stems and growing leaves, bract and bracteole-like leaflets absent; peduncle 1–2.5 cm; rachis glabrous to sparsely pubescent like the stems; pedicels 0.7–1.5 cm, articulate in the lower half, with small glandular trichomes. **Calyx** 0.4–1.0 cm in diameter, pubescent with long and short, simple, uniseriate trichomes; tube less than 0.5 mm; lobes to 5 mm, linear, the apex acute. **Corolla** 1.6–3 cm in diameter, bright yellow; tube minute, the corolla often divided almost to the base; lobes 0.7–1.2 × 0.2–0.5 cm, four times as long as wide, narrowly lanceolate, strongly reflexed at anthesis. **Staminal column** 6–8 mm, narrowly cone-shaped; filaments 1–2.5 mm; anthers 3.5–5 mm, the sterile tip approximately half the total anther length. **Ovary** conical, minutely glandular-villous; style 7–10 mm, usually exserted from the staminal column; stigma minute. **Fruits** (0.8–)1.1–1.6 cm in diameter, globose and 2-locular, glabrescent and becoming bright red at maturity; calyx lobes in fruit accrescent, 0.6–1.3 × 0.15–0.25 cm, strongly reflexed. **Seeds** (15–)50 (–80) per fruit, *c.* 2–3 × 1–1.5 mm, beaked; testa appearing hairy over entire surface and winged with the elongate lateral cell walls; dry seed weight *c.* 1 mg.

DISTRIBUTION. Coastal South America from Ecuador to Chile at low elevations; in the Galápagos mostly in disturbed areas on Santa Cruz, Isabela and San Cristóbal.

COMMON NAMES. Tomatillo, current tomato.

USES. Edible and sweet to taste. Cultivated and used by plant breeders to improve commercial cultivars of *Solanum lycopersicum*.

REPRESENTATIVE SPECIMENS EXAMINED.

ECUADOR. Galápagos. Isabela: Villamil, 1985, *TGRC accession LA2857* (TGRC seed bank); just outside Villamil, by El Lagoon del Manzanillo, 16 m, 00°55′85″S, 90°58′68″W, 25 July 2000 *Darwin, S. & Rosero 371*(BM). **San Cristóbal**: Puerto Baquerizo Moreno, E. side of town by Bethel School, roadside, 40 m, 00°54′37″S, 89°36′38″W, 5 August 2000, *Darwin, S. & Carrera 379* (BM, CDS), Puerto Baquerizo Moreno, E. side of town by Bethel School, roadside, 40 m, 00°54′37″S, 89°36′38″W, 5 August 2000, *Darwin, S. & Carrera 380* (BM, CDS). **Santa Cruz**: Bella Vista village, road S./SE of village, roadside lava, 200 m, 00°41′70″S, 90°19′43″W, 21 June 2000, *Darwin, S. 103* (BM, CDS, QCA, QCNE); between Puerto Ayora and Bella Vista, W. of main road, old basura site, disused rubbish dump, 125 m, 00°43′09″S, 90°19′81″W, 22 June 2000, *Darwin, S. et al. 104* (BM, CDS, QCNE); between Puerto Ayora and Bella Vista, W. of main road, old basura site, disused rubbish dump, 125 m, 00°43′09″S, 90°19′81″W, 22 June 2000, *Darwin, S. et al. 109* (BM, CDS, QCNE); between Los Gemelos and Canal, W. side of main road, the 'new' basura, disturbed ground around refuse area, 314 m, 00°35′04″S, 90°21′37″W, 22 June 2000, *Darwin, S. et al. 114* (BM, CDS, QCA, QCNE); between Los Gemelos and Canal, W. side of main road, the 'new' basura, disturbed ground around refuse area, 314 m, 00°35′04″S, 90°21′37″W, 22 June 2000, *Darwin, S. et al. 125* (CDS); Puerto Ayora, roadside, 40 m, 9 July 2000, *Darwin, S. 277* (BM, CDS); Puerto Ayora, roadside, 40 m, 9 July 2000, *Darwin, S. 278* (BM, CDS); El Chato Tortoise Reserve, by the lake, 200 m, 00°40′38″S, 90°26′32″W, 7 August 2000, *Darwin, S. et al. 400* (QCNE); El Chato Tortoise Reserve, by lake, 200 m, 00°40′38″S, 90°26′32″W, 7 August 2000, *Darwin, S. et al. 401* (BM); El Chato Tortoise Reserve, by lake, 200 m, 00°40′38″S, 90°26′32″W, *Darwin, S. et al. 402* (BM, CDS); El Chato Tortoise Reserve, by lake, 200 m, 00°40′38″S,

90°26′32″W, 7 August 2000, *Darwin, S. et al. 403* (QCNE); Mino Granilla Roja, 565 m, 0°36′56.6″S, 90°21′53.9″W, 26 July 2001, *Pozo & Herrera 2* (CDS).

Solanum pimpinellifolium can be distinguished from the other three species examined here by its markedly stellate flowers with narrowly lanceolate corolla lobes; the other three taxa have much more deltate lobes with longer corolla tubes (e.g. corolla divided approximately halfway rather than almost all the way to the base). The leaf margins are more entire than any of the other species and the bright red fruit are much smaller than those of *S. lycopersicum*. The dry seeds of *S. pimpinellifolium* are about double the weight of either of the two endemic tomatoes and about half the weight of *S. lycopersicum*.

It is not clear from the literature when the 'true' red-fruited *S. pimpinellifolium* and *S. lycopersicum* were first introduced to the Galápagos. The situation is made all the more complicated by earlier authors recognizing the native Galápagos taxa as varieties of *S. pimpinellifolium* and *S. lycopersicum*.

Müller (1940) cited several Galápagos specimens as *S. pimpinellifolium*; most are of the 'Academy Bay' morph of *S. cheesmaniae*, save one (*Stewart 3380*). The leaf morphology of this specimen fits extremely well with the Linnaean type of *S. pimpinellifolium*. It is also similar to plants of *S. pimpinellifolium* currently found in the Galápagos. This specimen, however, is enigmatic in that it has deltate corolla lobes and is more glabrous than Galápagos plants of *S. pimpinellifolium*, suggesting it belongs to the 'Academy Bay' morph of *S. cheesmaniae*. *Stewart 3380* lacks mature fruit, precluding firm conclusions.

The distribution of *S. pimpinellifolium* in Galápagos to date is largely in disturbed areas, but is documented in detail to enable spread to be monitored. On Santa Cruz the species is found in Puerto Ayora (Academy Bay), Bella Vista, Los Gemelos on the borders of the cloud forest and the El Chato Tortoise Reserve. It is also found along roadsides throughout the island, quarries and rubbish dumps. In some areas, for example in the El Chato Tortoise Reserve, it covers large areas of ground to the exclusion of other plants. On Isabela *S. pimpinellifolium* occurs near Villamil and along the road towards San Tomás and at El Lagoon del Manzanillo. On San Cristóbal the species has been collected only near the town of Puerto Baquerizo Moreno. The first unequivocal record of *S. pimpinellifolium* in Galápagos is a TGRC accession (LA2857) from Villamil collected in 1985 (Chetelat in litt., 2002); however, the species may have been introduced to the islands before 1905 if *Stewart 3380* is indeed from a plant of *S. pimpinellifolium*. Specimens collected by S. Darwin from Santa Cruz are therefore the earliest herbarium specimens that we can confirm as *S. pimpinellifolium*.

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Exsiccatae

Alphabetical by collector, all specimens examined.

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Aldaz, I. s.n. (cheesmaniae); 350 (cheesmaniae).

Andersson, N.J. s.n. (cheesmaniae); s.n. (galapagense).

Baur, G. s.n. (galapagense); 189 (galapagense).

Belt, R.L. s.n. (galapagense).

Bentley, P.S. 342 (cheesmaniae*).

Bowman, R.I. s.n. (galapagense); s.n. (galapagense); 40 (cheesmaniae*); 119 (cheesmaniae); 120 (cheesmaniae*); 121 (cheesmaniae*).

Castro, M. s.n. (galapagense); s.n. (galapagense); s.n. (cheesmaniae); s.n. (galapagense); s.n. (galapagense).

Cavagnero, D. 25 (galapagense).

Cheesman, L.E. in Riley 403 (cheesmaniae).

Clark, D.A. & Clark, D.B. 344 (cheesmaniae).

D'Arcy, W.G. 17754 (galapagense).

Darwin, C. s.n. (galapagense); s.n. (cheesmaniae); s.n. (cheesmaniae); s.n. (cheesmaniae*).

Darwin, S. 100 (pimpinellifolium); 101 (pimpinellifolium); 103 (pimpinellifolium); 276 (pimpinellifolium); 277 (pimpinellifolium); 278 (pimpinellifolium); 289 (lycopersicum); 304 (lycopersicum); 305 (lycopersicum).

Darwin, S. & Carrera, P. 378 (pimpinellifolium); 379 (pimpinellifolium); 380 (pimpinellifolium); 381 (lycopersicum); 384 (pimpinellifolium); 386 (pimpinellifolium).

Darwin, S., Chavez, J., Gardener, M. & Rejmanek, M. 290 (cheesmaniae*); 291 (galapagense); 292 (galapagense); 293 (galapagense); 294 (galapagense); 295 (galapagense); 296 (galapagense); 298 (galapagense); 299 (galapagense); 302 (lycopersicum); 303 (lycopersicum).

Darwin, S., Fitter, D. & Fitter, T. 195 (pimpinellifolium); 196 (pimpinellifolium); 197 (pimpinellifolium); 200 (pimpinellifolium); 201 (cheesmaniae); 202 (cheesmaniae); 203 (cheesmaniae); 204 (cheesmaniae); 205 (cheesmaniae); 206 (cheesmaniae); 207 (cheesmaniae); 208 (cheesmaniae); 209 (cheesmaniae); 210 (cheesmaniae); 211 (cheesmaniae); 280 (pimpinellifolium); 282 (pimpinellifolium); 283 (pimpinellifolium); 284 (pimpinellifolium); 285 (pimpinellifolium); 286 (pimpinellifolium).

Darwin, S., Fitter, D., Fitter, T. & Appleton, G. 212 (cheesmaniae); 213 (cheesmaniae); 214 (cheesmaniae); 215 (cheesmaniae); 216 (cheesmaniae); 217 (cheesmaniae); 218 (cheesmaniae); 219 (cheesmaniae); 220 (cheesmaniae); 221 (cheesmaniae); 222 (cheesmaniae); 223 (cheesmaniae); 224 (cheesmaniae); 225 (cheesmaniae); 226 (cheesmaniae); 227 (cheesmaniae); 228 (cheesmaniae); 229 (cheesmaniae); 230 (cheesmaniae); 231 (cheesmaniae); 232 (cheesmaniae); 233 (cheesmaniae); 234 (cheesmaniae); 235 (cheesmaniae); 236 (cheesmaniae); 238 (cheesmaniae); 239 (cheesmaniae); 240 (cheesmaniae); 241 (cheesmaniae); 243 (cheesmaniae); 244 (cheesmaniae); 245 (cheesmaniae); 246 (cheesmaniae); 247 (cheesmaniae); 248 (cheesmaniae); 249 (cheesmaniae); 250 (cheesmaniae); 251 (cheesmaniae); 252 (cheesmaniae); 253 (cheesmaniae); 254 (cheesmaniae); 255 (cheesmaniae); 256 (cheesmaniae); 257 (cheesmaniae); 258 (cheesmaniae); 259 (cheesmaniae); 261 (cheesmaniae); 262 (cheesmaniae); 263 (cheesmaniae); 264 (cheesmaniae); 265 (cheesmaniae); 266 (cheesmaniae); 267 (cheesmaniae); 268 (cheesmaniae); 269 (cheesmaniae); 270 (cheesmaniae); 271 (cheesmaniae); 272 (cheesmaniae); 273 (cheesmaniae); 274 (cheesmaniae); 260 (cheesmaniae).

Darwin, S., Gardener, M. & Callebaut, J. 388 (pimpinellifolium); 389 (pimpinellifolium); 390 (pimpinellifolium); 391 (pimpinellifolium); 392 (pimpinellifolium); 393 (pimpinellifolium); 394 (pimpinellifolium); 395 (pimpinellifolium); 396 (pimpinellifolium); 397 (pimpinellifolium); 398 (pimpinellifolium); 399 (pimpinellifolium); 400 (pimpinellifolium); 401 (pimpinellifolium); 402 (pimpinellifolium); 403 (pimpinellifolium); 405 (pimpinellifolium); 407 (pimpinellifolium); 410 (pimpinellifolium); 411 (pimpinellifolium); 412 (pimpinellifolium); 413 (pimpinellifolium); 414 (pimpinellifolium); 415 (pimpinellifolium); 416 (pimpinellifolium); 417 (pimpinellifolium); 418 (pimpinellifolium); 419 (pimpinellifolium); 421 (pimpinellifolium); 422 (pimpinellifolium); 423 (pimpinellifolium); 424 (pimpinellifolium); 425 (pimpinellifolium); 426 (pimpinellifolium); 427 (pimpinellifolium).

Darwin, S. & Robayo, J. 377 (pimpinellifolium).

Darwin, S. & Rosero, P. 354 (lycopersicum); 355 (lycopersicum); 356 (lycopersicum); 357 (lycopersicum); 358 (lycopersicum); 359 (lycopersicum); 360 (lycopersicum); 364 (galapagense); 365 (cheesmaniae*); 366 (cheesmaniae*); 367 (galapagense); 372 (galapagense); 373 (galapagense); 374 (galapagense); 375 (galapagense); 376 (lycopersicum).

Darwin, S. & Schultz, A. 127 (galapagense); 132 (galapagense); 133 (galapagense); 134 (galapagense); 135 (galapagense); 138 (galapagense); 139 (galapagense); 140 (galapagense); 141 (galapagense); 142 (galapagense); 143 (galapagense); 144 (galapagense); 145 (galapagense); 146 (galapagense); 147 (galapagense); 148 (galapagense); 149 (galapagense); 150 (galapagense); 151 (galapagense); 152 (galapagense); 153 (galapagense); 154 (galapagense); 155 (galapagense); 156 (galapagense); 157 (galapagense); 158 (galapagense); 159 (galapagense); 160 (galapagense); 161 (galapagense); 162 (galapagense); 163 (galapagense); 164 (galapagense); 165 (galapagense); 166 (galapagense); 167 (galapagense); 168 (galapagense); 169 (galapagense); 170 (galapagense); 171 (galapagense); 173 (galapagense); 174 (galapagense); 175 (galapagense); 176 (galapagense); 177 (galapagense); 179 (galapagense); 180 (galapagense); 181 (galapagense); 182 (galapagense); 183 (galapagense); 184 (galapagense); 185 (galapagense); 186 (galapagense); 187 (galapagense); 188 (galapagense); 189 (galapagense); 190 (galapagense); 191 (galapagense); 192 (galapagense); 193 (galapagense); 194 (galapagense).

Darwin, S., Tye, A., Jäger, H., Callebaut, J. & Schultz, A. 104 (pimpinellifolium); 105 (pimpinellifolium); 106 (pimpinellifolium); 108 (pimpinellifolium); 109 (pimpinellifolium); 110 (pimpinellifolium); 111 (pimpinellifolium); 112 (pimpinellifolium); 113 (pimpinellifolium); 114 (pimpinellifolium); 124 (pimpinellifolium); 125 (pimpinellifolium); 126 (pimpinellifolium).

Day, D. 287 (galapagense).

de Vries, T. s.n. (galapagense); 1227 (cheesmaniae).

DeRoy, A. & DeRoy, J. 11 (galapagense).

Eliasson, U. & Eliasson, I. 201 (cheesmaniae*); 656 (galapagense); 1106 (galapagense); 1643 (cheesmaniae*); 2207 (cheesmaniae*).

Fagerlind, F. & Wibom, G. 3070 (cheesmaniae*); 3110 (cheesmaniae*); 3464 (galapagense); 3471 (galapagense).

Fosberg, F.R. 45012 (cheesmaniae*).

Hamann, M. & Hamann, O. 193 (cheesmaniae* (sheet at C), mixed collection with galapagense (sheet at CDS)); 194 (galapagense); 213 (cheesmaniae*); 267 (cheesmaniae*); 269 (cheesmaniae*); 444 (cheesmaniae); 1698 (cheesmaniae*); 1729 (galapagense); 1801 (cheesmaniae*); 2483 (galapagense).

Hamann, O. & Seberg, O. 1771 (cheesmaniae).

Harling, G. 5288 (cheesmaniae); 5371 (cheesmaniae); 5476 (cheesmaniae).

Herndactmes, C. s.n. (cheesmaniae).

Howell, J.T. 10012 (galapagense); 8573 (lycopersicum); 9096 (cheesmaniae*); 9427 (cheesmaniae*); 9447 (cheesmaniae); 9617 (cheesmaniae); 9701 (galapagense); 9753 (galapagense).

Huttel, C. 495 (cheesmaniae*); 1597 (cheesmaniae); 2735 (cheesmaniae).

Jaeger, H. & Leuchten, S. 9068 (galapagense).

Jaramillo, P. 1052 (galapagense).

Lawesson, J.E. 3017 (cheesmaniae*); 3080 (cheesmaniae*); 2638 (galapagense); 3234 (galapagense).

Lévêque, R. 163 (galapagense).

Müller & Müller 2500 (cheesmaniae*).

Porter, D.M. s.n. (galapagense).

Pozo, P. & Herrera, H. 2 (pimpinellifolium).

Reeder, L.R. s.n. (galapagense).

Reeder ??& Chapy s.n. (galapagense).

Reeder, Wm. G. s.n. (galapagense).

Schimpff, H.J.F. 12 (cheesmaniae*).

Schmidt, A. & Schmidt, P. 2528 (galapagense).

Scouler, J. s.n. (galapagense).

Snell, H. 109 (galapagense).

Snodgrass, R.E. & Heller, E. 305 (galapagense); 399 (galapagense); 741 (galapagense); 843 (galapagense); 911 (galapagense); 928 (cheesmaniae)

Snow, A.W. s.n. (cheesmaniae); s.n. (galapagense); 297 (galapagense); 498 (cheesmaniae); 591 (galapagense).

Stewart, A. s.n. (galapagense); 3369 (galapagense); 3370 (galapagense); 3372 (galapagense); 3373 (galapagense); 3374 (cheesmaniae); 3375 (cheesmaniae); 3376 (cheesmaniae); 3377 (galapagense); 3378 (galapagense); 3379 (cheesmaniae).

Svenson, H.K. 281 (cheesmaniae*).

Touc, L.T. s.n. (galapagense); s.n. (cheesmaniae*); s.n. (galapagense); s.n. (cheesmaniae); s.n. (cheesmaniae); s.n. (galapagense); s.n. (galapagense).

van der Werff, H.H. 1265 (cheesmaniae); 2129 (galapagense).

Verdugo, A. 15 (galapagense).

Werner, D. 2541 (cheesmaniae*); 2552 (cheesmaniae).

Wiggins, I.L. & Porter, D.M. 296 (galapagense); 314 (galapagense); 604 (cheesmaniae*).

Chapter 3

Galápagos tomato genetics and hybridization

Introduction

This chapter has been divided into three sections. A general Materials and Methods was written for the whole chapter. A shorter Materials and Methods was included in each section for details that specifically applied to individual analyses or plants from that section.

Chapter 3a is an overall outline into the genetic diversity of Galápagos tomatoes using allozyme electrophoresis. This includes plants that were collected in the field by Sarah Darwin (SCD) in 2000 and 2002. Only those that were identified in the field as being morphologically pure were included here. However, several individual plants that were assumed to have been 'pure' in the field were subsequently discovered to be putative hybrids. They are included here, too.

Chapter 3b is a study of three different groups of hybrids in the Galápagos Islands, representing field collections by SCD in 2000 and 2002. These hybrids were primarily identified in the field using morphological characters. The different hybrid groups were then analysed using the allozyme markers that had been established in the first section for the pure species of tomatoes form the Galápagos Islands. This chapter has both, detailed morphometric and genetic analyses and a test of their congruency. These different hybrid groups included:

1) Hybrids between *S. cheesmaniae* x *S. galapagense* from El Lagoon de Manzanilla (Isla Isabela);
2) Hybrids between *S. pimpinellifolium* x *S. lycopersicum* at Puerto Baquerizo (Isla San Cristóbal);
3) A detailed study was undertaken of hybrid populations of *S. cheesmaniae* x *S. pimpinellifolium* found along the Baltra Road (Isla Santa Cruz).

Chapter 3c is an allozyme study of the seed bank accessions sourced from the Tomato Genetic Resource Center and Cornell seed bank.

General materials and methods for the chapter

Sampling

Material for allozyme electrophoresis was obtained from fresh leaf samples of *Solanum cheesmaniae, S. galapagense, S. pimpinellifolium* and *S. lycopersicum*; both from wild collected plants from the Galápagos Islands (ten samples) and from plants having been grown under greenhouse conditions at the Chelsea Physic Garden, London UK in 2000, 2000/2001 and 2003 (1247 samples).

Greenhouse raised tomato plants were grown from seeds obtained from three different sources:

1- Wild collected by Sarah C. Darwin (SCD) from the Galápagos Islands during fieldwork in June to August 2000 and September to November 2002);

2 – Seed accessions generously donated by the Tomato Genetic Resource Center based at UC Davis California (TGRC);

3 –Seed accessions kindly donated by the Cornell Seed Bank Collection (six accessions) (these collections were originally obtained from TGRC);

Accessions were divided into either populations or geographical regions. In total 93 populations and geographical regions were tested, of these, 55 populations were collected in the Galápagos Islands by SCD (in 2000 and 2002) and 38 geographical regions were sourced from the TGRC and Cornell seed banks. A plant population is defined here as a group of individuals which are within geographical proximity allowing the potential of cross breeding and from which individuals may be recruited. Where possible, the seed bank accessions were grouped together into geographical regions and these were in some cases

represented by accessions collected over a number of different years. However, many seed bank geographical regions were represented by a single accession. Populations collected from the same region but in different years were separated according the collection year.

A maximum number of plants were sampled from each wild locality, the largest number of plants per population was at the Basura (population 107), Baltra Road, Isla Santa Cruz. Here, 83 tomato plants were collected in total, represented by - 13 *S. cheesmaniae*, 35 *S. pimpinellifolium* and 35 *S. cheesmaniae* x *S. pimpinellifolium* hybrids. However in some areas a population was represented by a single plant. Each field-collected plant was described as an accession. All SCD field collected plant accessions were given a unique code in the field and locality information including longitude and latitude and altitude were recorded for each population (see Table 3.1).

The species circumscription described in Darwin *et al*. (2003) was used in the field and this was undertaken at the time of collection. Many field-collected individuals were found to be morphologically intermediate between two species and were thus described as putative hybrids. These putative hybrids were analyzed as separate groups in their own right. Some accessions were found to be morphologically 'pure' and then subsequently during analysis found to have alleles from other taxa, these plants remained in their original morphologically designated species. Herbarium voucher specimens were made from all samples, where possible.

The TGRC and Cornell seed accessions could not be described as populations because each accession is represented by single set of seeds. In some cases more than one TGRC seed accession has been collected from the same locality but in different years. TGRC collections that were the same area were pooled together and described as occurring in the same geographical region.

Each TGRC plant accession has a unique *Lycopersicum* accession (LA) number, the species name, collection date and locality. During this research each of the TGRC LA numbers was given a unique SCD code (SCD 001-069) to fit in and not to be confused with the other SCD accession numbers. The

TGRC species circumscription was upheld for all accessions with the exception of that for LA3123 (SCD 069, see discussion below).

Germination and growing conditions

Tomato plants were raised from seed in the Greenhouses at the Chelsea Physic Garden, London. Seed dormancy was broken using a method devised by the TGRC (Rick and Borgnino, http://tgrc.ucdavis.edu/seed_germ.aspx). This involved soaking seeds in a diluted solution of sodium hypochlorite (household bleach) with the following modifications:

One seed, to several seeds (depending on availability) of each accession were placed into small coffee filter bags (numbered with the individual SCD accession number) and then placed into a tray containing the dilution of sodium hypochlorite. This method ensured that up to 20 different seed accessions could be treated simultaneously without risk of different seed accessions mixing. Seeds were then transferred onto numbered moist blotting paper and into individual Petri dishes. After approximately seven days, all un-germinated seeds were re-treated with sodium hypochlorite using the same method with the coffee filter bags. This re-treatment was repeated until germination took place to a maximum of four times.

After germination the seedlings were transferred from the numbered Petri dishes into numbered compartmentalised seed trays (well size 80mm) containing John Innes loam (7parts loam, 3 parts coir and 2 parts grit and bark). These trays were placed either into the heated ‘pyramid greenhouse’ (during the winter months) or ‘pit’ greenhouse at the Chelsea Physic Garden. Each seedling was numbered with the accession number followed by a number to indicate the germination order and indicating that it was an offspring of the original seed collected. For example accession number 147-1 would indicate SCD field accession number 147 and individual offspring plant number 1. This also gave system for different generations to be numbered. For example the first progeny of 147-1 would be 147-1-1.

The second set of accessions to be grown 2000/2001 were treated during the British winter. After germination the plants were grown on in the heated

'pyramid greenhouse'. Light levels were augmented and extended with the addition of artificial lighting for four hours a day; two hours in the morning (1000 to 1200 hours) and two hours in the afternoon (1600 to 1800 hours).

Electrophoresis

Allozyme electrophoresis was carried out using the NHM Botany Department allozyme electrophoresis laboratory protocol (see Vogel *et al*., 1999). The following enzyme systems were informative and could be analyzed for locus and allelic variation: diaphorase (DIA, 1.6.99), isocitrate dehydrogenase (IDH, 1.1.1.42), malate dehydrogenase (MDH, 1.1.1.37), phosphglucoisomerase (PGI, 5.3.1.9), phosphoglucomutase (PGM, 5.4.2.2), 6-phosphogluconate dehydrogenase (6-PGD, 1.1.1.44) and triose-phosphate isomerasee (TPI 5.3.11). Band homologies were elucidated by running samples side-by-side and twenty loci were resolved. The methods used for allozyme electrophoresis are described in full in Vogel *et al.* (1999). Tasks in the laboratory were undertaken by a team and were divided amongst the following people - Johannes Vogel, Michael Grundmann, Stephen Russell, Harriet Hunt, and myself.

Genetic diversity analysis

Analysis was undertaken on two main data sets – the bulk and the individual collection data sets (*sensu* DeWalt and Hamrick, 2004).

The 'bulk' collection data set represented all plants that were grown in each accession and each accession was represented on average by three plants grown in the greenhouse. For some analyses a mean result was taken from the groups of offspring to represent the field-collected individuals.

Selecting a single individual plant per accession from the bulk data set randomly created the 'Individual' collection data set. This Random selection was undertaken on all accessions that were represented by more than one individual plant (EXCEL random generated numbers between 0 – 1).

Coding

Plants were divided into populations/geographic regions. Each island and each population were assigned numbers. In addition to this the collection year was included represented by the last two digits for both the TGRC and SCD collections. For example a population on Isla Santa Cruz (island 1) rubbish dump population (07) and plants collected in (2002) would become 10702, see Table 3.1. In addition to this each accession retained its unique SCD code so that it could be identified.

Allele frequency tables and allele presence tables

The SCD individual collection and bulk collection and the seed bank individual and bulk collections were compared using F-statistics. It was established that the SCD individual collection should be analysed rather than the bulk collection. This was because each individual would represent a single offspring from a single accession collected in the field. The TGRC seed bank accessions are formed from a collection of seeds harvested from fruits of many plants assigned to an individual accession. For this reason the bulk collection data set was used for analysis of the seed bank collections.

Allozyme frequency tables were calculated using POPGENE 3.1 (Yeh *et* al., 1999). This was using the individual data set for the SCD collections. Frequency tables were created for all the tomatoes species found in the Galápagos Islands. Additional frequency tables were tables were created for each of the three hybrid populations.

The bulk data sets were used to compile several tables of allele presence in both SCD collections and seed bank collections. Frequencies were less relevant with the seed bank accessions due to the small sample sizes at each geographic locality and the time differences between each collection).

Statistical analysis was undertaken in POPGENE (3.1) to establish within and between population diversity and estimate departure from Hardy–Weinberg equilibrium. Different groupings were analysed for the SCD individual collections – 1) each taxa, 2) each island within each taxa, 3) the putative *S. cheesmaniae x S. pimpinellifolium* from the Baltra Road on Isla Santa Cruz, 4)

the putative *S. cheesmaniae x S. galapagense* hybrids on Isla Isabela 5) the putative *S. pimpinellifolium* x *S. lycopersicum* hybrids on Isla San Cristóbal. Different groupings were analysed for the TGRC bulk collection: 1) a summary of the different taxa, 2) the different islands within each taxa. Neutrality tests were undertaken in POPGENE in order to detect evidence of selection in the loci.

F-statistics were undertaken for all the collections SCD bulk and individual and seed bank bulk and individual. These were calculated to elucidate departure from Hardy-Weinberg equilibrium.

Statistical analysis with STRUCTURE 2.2

STRUCTURE 2.2 (Pritchard and Wen, 2003) was undertaken on two sets of data. 1) The pure Galápagos tomato populations as defined by morphology in the field and 2) on the putative hybrid populations of *S. cheesmaniae x S. pimpinellifolium* from the Baltra Road on Isla Santa Cruz and nearby pure populations of both *S. cheesmaniae* and *S. pimpinellifolium* were included in this analysis for a comparison.

A minimum burn-in of 400,000 and 2000,000 iteration cycles (400k, 2000k) (or an excess of these) was used with each analysis. This was established after confirming that the results from using longer cycles did not give different results.

The following STRUCTURE analyses were carried out using the following assumptions: admixture ancestry model and allele frequency correlated.

Table 3.1. Population localities, names, numbers, coordinates and altitudes

Island Name and No.		SCD	TGRC	Cornell	Pop. Code	Population Name	Latitude And Longitude	Alt. (approx.)
Baltra	**0**	c	c		001	North Coast	00˚24'55"S 90˚17'21"W	<5m
Santa Cruz	**1**	c			101	North Coast	00˚33'35"S90˚10'55"W	<5m
		c			102	North Coast	00˚32'29"S90˚12'50" W	<5m
		c			103	North Coast	00˚31'45"S90˚14'01" W	<5m
		c			104	North Coast	00˚28'55"S90˚15'00"W	<5m
		ch			105	"Zone of Birds"	00˚31'55"S90˚18'43"W	100m
		ch			106	Mino Granillo Negro	00˚34'25"S90˚19'59"W	330m
		cph			107	"Basura"	00˚35'04"S90˚21'37"W	341m
		ph			108	Mino Granillo Rojo	00˚37'02"S90˚22'02"W	600m
		p	c		109	Los Gemelos	00˚37'31"S90˚23'02"W	595m
		p			110	El Chato	00˚40'38"S90˚26'32"W	180m
		p			111	Bella Vista	00˚41'27" S 90˚19'47"W	/
		p			112	Road to Garapaterra Beach	00˚40'07"S90˚16'00"W	250m
		p			113	Road to Garapaterra Beach	00˚41'59"S90˚13'28"W	/
		p			114	Puerto Ayora	00˚44'22"S90˚18'42"W	20m
		p			115	Old Basura	00˚43'09"S90˚19'44"W	125m
			c		117	Punta Nuñez	/	/
			l		118	Playa to Bella Vista	/	/
			c		119	El Cascajo	/	/
			p		120	"Towards Baltra"	/	/
Santa Fé	**2**	c			201	Centre barranco	00˚48'54"S90˚04'39"W	120m
			c		202	East landing	/	/
Isabela	**3**	cg cxg l	cg		301	Villamil & Ponds and El lagoon	00˚57'27"S90˚57'58W	20m
		cg l	c		302	San Tomas	00˚51'25"S90˚01'54"W	280m
		g			303	San Pedro	01˚01'53"S91˚13'22"W	<20m
		c	c		304	Caleta Iguana & Punta Essex	00˚58'57"S91˚26'44"W	35m
			c		305	Cerro Azul	/	
		cg			306	Punta Cristóbal	00˚52'28"S91˚30'23"W	8m
		c			307	5k W Caleta Webb	00˚43'57"S91˚21'06"W	5m
		g	cg		308	Playa Tortuga Negra, Tagus Cove, Caleta Tortuga & Caleta Negra	00˚12'44"S91˚23'45"W	<30m
		g			309	East of Volcán Ecuador	/	/
			g		310	Cape Berkeley	/	/
			c		312	Punta Albemarle & "Far North"	/	/
			g		313	Alcedo	/	/
			g		314	"Cowley Bay"	/	/
				g	315	"Punta Ecuador"	/	/
Floreana	**4**		g		401	Corona del Diablo	/	/
			g		402	Gardner	/	/
Rábida	**5**	g	g		501	"North Side"	/	/
Fernandina	**6**		g	c	601	Crater Lake & Crater Rim	/	/
		g			602	Cabo Douglas	?	/
		g			603	Cabo Hammond	/	/
			c	g	604	Punta Espinosa	/	/
		g			605	5k E Cape Douglas	00˚17'53"S91˚37'41"W	<15m
		cg			606	Los Túneles	00˚16'55"S91˚30'50"W	10m
			c		607	"North Side"	/	/
			c		608	"Low Elevations, SE side"	/	/

Table 3.1 continued

Island Name and No.	SCD	TGRC	Cornell	Pop. Code	Population Name	Latitude And Longitude	Alt. (approx..)
Pinzón **7**	c			701	Crater base	00˚36'35"S90˚40'06"W	160m
	g	g		702	North Coast	00˚35'27"S90˚40'39"W	<15m
Santiago **8**	g	g		801	Bartolomé	00˚16'57"S90˚33'32"W	<15m
		g		802	Cape Trenton	/	/
			g	803	James Bay	/	/
		g		804	Trenton Island	/	/
San Cristóbal **9**	p pxl	c		901	Puerto Baquerizo Moreno & Wreck Bay	00˚54'37"S89˚36'38"W	<50m
			c	902	Opposite Islas los Lobos	/	/

Legend

Taxon codes in table 3.1

c = *Solanum cheesmaniae*

p = *Solanum pimpinellifolium*

g = *Solanum galapagense*

h = Hybrid between *Solanum cheesmaniae* and *S. pimpinellifolium*

l = *Solanum lycopersicum*

x = hybrid between any two other species

Chapter 3a

Genetic diversity in tomatoes from the Galápagos Islands

Introduction

This is a large genetic study involving the four species of tomatoes (*Solanum* spp., Solanaceae) found in the Galápagos Islands. There are two endemic species - *Solanum cheesmaniae* (L. Riley) Fosberg*, S. galapagense* S. C. Darwin and Peralta and two introduced species - *S. lycopersicum* L. and *S. pimpinellifolium* L. Solanaceae.

The genetic structure of plant populations is dependant upon several factors - mutation, selection, drift, breeding systems and stochastic events (Vogel *et al.,* 1999). Genetic variation is created by mutation and eroded by drift and selection (Lowe *et al*., 2004). The smaller the population the more likely it is that a stochastic event will lead to genetic drift leaving the population with a different allele frequency to the original population (Lowe *et al*., 2004). Founder effects (colonisation by a few individuals) or bottlenecks can lead to reduction in population diversity due to, for example, habitat fragmentation (Lowe *et al*., 2004), in the case of Galápagos tomatoes, perhaps by volcanic eruptions and goat predation.

The Galápagos Islands lie about 1000 km off the west coast of Ecuador. The islands are volcanic in origin and are between 2 - 6.5 million years old. The oldest islands are on the east of the archipelago (Geist, 1996). There are 13 large islands (over 10 km^2) and over 40 officially named smaller islands, islets and emergent rocks (see Fig. 1).

Five islands in the Galápagos archipelago have permanent human populations – Islas Floreana, San Cristóbal, Isabela, Santa Cruz and Baltra. Isla Santiago,

although currently uninhabited, in the past had a settlement at Bahía James (Wiggins and Porter, 1971). The islands although discovered by Europeans in 1535 (Perry, 1972), were only permanently settled in 1830. The 2001 census estimated the resident population at 18,640 (http://www.citypopulation.de/Ecuador.html#Land). The current population figure probably lies at around 30,000 (G. Watkins pers. comm., 2008)

About 40% of the native flowering plants of the Galápagos Islands are endemic. The total number of native vascular plants is approximately 596 taxa; with 372 natives and 224 endemics (Lawesson, 1990b). In common with other oceanic archipelagos, the Galápagos Islands suffer from the effects of introduced non-native animals and plants (Loope *et al*., 1988). Humans have brought these in either accidentally or intentionally. As is often the case, the early check lists of Galápagos plants only included native and endemic taxa. Only since the 1960s naturalized plants have been included and the mid 1980s saw the inclusion of cultivated plants in checklists in Galápagos (Tye, 2006). Lawesson (1990a) estimated that there were approximately 260 introduced plants. More recent analyses show that the number of introduced plants is both higher and rising. A recent partial survey of the islands estimated over 550 non-native plant taxa (Tye, 2006) the number is likely to be considerably higher even than this.

The genus *Solanum* contains several important crop plants, for example potatoes, tobaccos and tomatoes. Wild tomatoes occur in South America, the centre of diversity being the western slopes of the Central Andes. There are 13 species of tomato (four of which are considered edible), the cultivated tomato *S. lycopersicum* and 12 wild relatives (Peralta *et al*., 2008). Linnaeus originally placed tomatoes in the genus *Solanum* in 1753. Later Philip Miller, a contemporary of Linnaeus', reclassified the tomatoes into the genus *Lycopersicon* (which at the time included the potato). Following both morphological studies (Child, 1990; Spooner *et al*., 1993; Bohs, 1994, 1995; Peralta *et al.*, 2008) and molecular studies (Spooner *et al.,* 1993; Bohs & Olmstead, 1997, 1999: Peralta & Spooner, 2001) most taxonomists regard tomatoes as *Solanum* as they are deeply nested within this genus and are sister to the potatoes. However some of the plant breeding community still refers to

tomatoes as *Lycopersicon*, largely for practical reasons (Peralta *et al*., 2008). I recognise the tomatoes as belonging to the genus *Solanum*.

The tomatoes from the Galápagos Islands were first described by Hooker (1847), in his enumeration of the Galápagos plants, as parts or forms of existing tomato species as '*Lycopersicum pimpinellifolium*', '*L. esculentum* var. *minor*' and '*L. peruvianum* var. *parviflorum*'. The inclusion of tomatoes (and most of the other species found in Hooker, 1847) was based on Charles Darwin's Galápagos plant collections made in 1835 during the voyage of the Beagle. Since then, Galápagos tomatoes have been variously classified both by taxonomists and plant breeders and there has been some controversy as to how many and to which species the Galápagos tomato taxa belong. In 1925, L.E. Riley segregated the Galápagos tomato into a distinct species – '*Lycopersicum cheesmanii*' (=*Solanum cheesmaniae*). In the *Flora of the Galápagos Islands* (Wiggins and Porter, 1971) Charles Rick (1971) recognised two forms of '*L. cheesmanii*'. '*Lycopersicon cheesmanii* forma *minor*' (=*S. galapagense*) and '*L. cheesmanii* forma *typicum*' (=*S. cheesmaniae*). (See Darwin *et al.,* 2003 for a detailed historical account of the classification of the tomatoes in the Galápagos Islands).

In 2003, Darwin *et al*., described the presence, in the Galápagos Islands, of two species of endemic tomato *S. cheesmaniae* Riley and *S. galapagense* Darwin and Peralta, (the latter described as new) and two species of introduced tomato *S. pimpinellifolium* L. and *S. lycopersicum* L. which were probably introduced by settlers during the twentieth Century. These four species of tomatoes that are found in the Galapagos Islands represent all the edible species and four out of the 13 species of tomatoes that have been described by science.

Within the Galápagos Archipelago tomatoes are growing on 19 different islands and islets (see Chapter 2). Only on two islands, Islas Fernandina and Isabela, do the endemic taxa, *S. cheesmaniae* and *S. galapagense*, currently grow sympatrically (Rick, 1971 and Darwin *et al*., 2003). Both endemic species are also found on Isla Pinzón but in allopatry - *S. galapagense* as a coastal plant on the north of this island and *S. cheesmaniae* at the base of an extinct volcanic crater.

Both endemic taxa have wide ecological amplitudes on the islands. They were collected along coastal lava within a few meters of the high tide mark (Darwin *et al*., 2003) and they both can also be found growing inland at higher elevations. *Solanum cheesmaniae* has been collected from the crater rim of Isla Fernandina at over 1200m (TGRC LA1427). *Solanum galapagense* although more commonly found as a coastal plant, has also been collected at higher elevations from the crater at Fernandina (TGRC LA483 and LA530).

The endemic Galápagos tomatoes are described as being self-compatible (Easlon and Richards 2009) predominantly self-pollinating. Tomato flowers are 'buzz pollinated' by bees this is achieved by specialised vibrations of the thoracic flight muscles, (Buchman, 1993; Knapp, 1986). This vibration prompts pollen dehiscence; the pollen is then ejected from staminal column towards the ventral surface of the bee. In the Galápagos Islands there is only one species of bee - the endemic carpenter bee - *Xylocopa darwini* (McMullen, 1990). It has also been suggested that Galápagos tomatoes may be wind pollinated (McMullen and Close, 1993). While the wind may assist self-pollination by movement (in much the same way that you can shake tomato flowers in a green house) it seems unlikely that cross-pollination, in any quantity, would take place by this method.

The ripe fruits of the endemic Galápagos tomatoes are eaten and dispersed by the endemic Galápagos giant tortoise (*Geochelone elephantopus*). Rick and Bowman (1961) found that the strong seed dormancy was greatly reduced if the seeds had passed through the gut of a tortoise. It has also been suggested that other animals in the Galápagos also eat tomatoes, for example - land iguanas (*Conolphus spp*.), Galápagos mockingbirds (*Mimus spp*.) and that goats (*Capra hircus*) might also be responsible for seed dispersal (Rick and Bowman, 1961).

Solanum lycopersicum is the commonly cultivated species of tomato around the world whereas *S. pimpinellifolium* is grown as an occasional home garden plant (Rick, 1984) and is commonly found growing wild in Ecuador. *Solanum lycopersicum* is found on four islands in the Galápagos Islands: Islas Santa Cruz, San Cristóbal, Isabela and Floreana. During fieldwork in 2000 and 2002 it

was growing in cultivation both as a crop plant and in vegetable gardens on Isla Isabela. *Solanum lycopersicum* was also found growing feral (*sensu* Richardson *et al*., 2000) as a garden/farm escape and as road side plant but never far from human habitations. Humans may disperse *Solanum pimpinellifolium* and *S. lycopersicum*, the introduced tomatoes, as locals, hunters and visitors eat tomatoes. Tomatoes in general are known to germinate well after having been through the human digestive system (for example http://www.ogtr.gov.au/pdf/volsys/pr127.pdf).

Solanum pimpinellifolium was found growing as a feral plant on the road side near sea level in Puerto Ayora to the margins of the cloud forest (600m), at the village of Bella Vista and along the roadside towards Garapaterra Beach. It was also found on the northern side of the island to an altitude of 320m along the Baltra Road on Santa Cruz and in several localities between. It has been recorded as an invasive plant (sensu Richardson *et al*., 2000) at El Chato Tortoise reserve (Darwin *et al*.' 2003), the Basura (rubbish dump) and at various locations along the Baltra Road for example the gravel mine - Mina Roja (see Table 1.4 for a summary of definitions for *S. pimpinellifolium* as an invasive plant species). Furthermore *S. pimpinellifolium* was found at Puerto Baquerizo on San Cristóbal. The current presence of *S. pimpinellifolium* on Isla Isabela remained unconfirmed although there is a TGRC accession (LA 2856) from Isla Isabela. However, this accession was not grown during this research.

There has been great interest in the endemic Galápagos tomatoes, not only because they are part of the islands' endemic flora but also for economic reasons. Galápagos tomatoes cross breed freely with the cultivated tomatoes and produce fertile offspring (Rick, 1979, Nuez *et al*., 2004). Being a close relative of such an important crop plant the tomato, has meant *S. cheesmaniae* and *S. galapagense* have been extensively investigated for useful characteristics to enhance the cultivated tomatoes. For example *S. cheesmaniae* plants from a single population at Puerto Ayora (Isla Santa Cruz) possessed an unusual pedicel morphology lacking the central articulation common to potatoes and tomatoes, a mutation known as 'jointless' (Rick, 1967). This allows the fruit to abscise at the base of the calyx which, when bred into the cultivated tomato, has made mechanised picking of tomatoes possible

(Rick, 1967). *Solanum galapagense* was also found to be more salt tolerant than more common cultivated tomatoes (Rush and Epstein, 1981). This allowed strains of drought resistant tomatoes to be developed and tomatoes cultivated in areas with high soil salinity. Other characters have been the subject of investigation for example the fruit soluble solids in the Galápagos tomatoes differing from domestic tomatoes. For further information see Darwin *et al*., (2003) and Nuez *et al*., (2004) and references therein.

Molecular techniques have been used to elucidate the relationship of the Galápagos tomatoes between each other and with other tomato species. Several molecular studies have included Galápagos tomatoes as part of a wider tomato studies for example, Rick and Fobes, (1975), Peralta and Spooner (2001), Nuez *et al*. (2004); Moyle (2008); Rodriguez *et al*. (2009).

Allozyme electrophoresis is a useful technique for comparing populations, as allozyme loci are numerous and co-dominant (Hamrick and Godt, 1990). Allozymes can be applied to estimate gene diversity and population structure as well as to find evidence of hybridization and introgression. They are assumed to be selectively neutral however there is some evidence of selection at some loci in some organisms (Lowe *et al*., 2004). Several molecular studies have used allozyme electrophoresis as a useful initial method for assessing genetic diversity within and between other island congenerics (see Crawford *et al*., 2006 and references therein).

In this chapter I want to address the following main questions:

1) Can the four different species of tomato from the Galápagos Islands, described using morphological characters, be discriminated using genetic markers and how does this correspond to the species circumscription outlined in Darwin *et al*. (2003)?
2) What genetic diversity is there within and between populations and taxa?
3) Is there any genetic evidence of hybridization between the taxa and is there congruence or incongruence between the genetic and morphological data?

Materials and methods

Taxa and population selection

This analysis was undertaken on offspring from plants that were collected during fieldwork in the Galápagos during 2000 and 2002. Populations that were regarded as 'pure' species, using morphological characters observed in the field only, were included in this analysis. 'Pure' species were selected here in order to establish natural wild diversity within each of the four species. Individual plants or populations identified in the field as hybrids were analyzed separately in Chapter 3b and plants grown from seed banks accessions were analyzed in Chapter 3c. The wild populations sampled for this investigation are shown in Table 3.2.1.

Allozyme electrophoresis

Seeds were collected from the Galápagos Islands in 2000 and 2002 by SCD. The greenhouse grown plants were grown from these seed. Allozyme electrophoresis was undertaken on fresh leaf material collected from these greenhouse grown plants.

Population diversity measures

A frequency table was constructed from data calculated in POPGENE (3.1). Within diversity measures, f-statistics and neutrality tests were calculated in POPGENE. Means diversity measures were calculated for the different taxa and islands using EXCEL.

STRUCTURE analysis

All populations of *S. lycopersicum* were excluded from the STRUCTURE (2.2) analysis.

Table 3.2.1 *Solanum cheesmaniae* populations used for population diversity analysis and STRUCTURE Note that the populations that are marked with * were excluded from the STRUCTURE (2.2) analysis.

Pop no.	Island	Pop name	Year
100	Baltra	North Coast	2000
101	Santa Cruz	North Coast	2000
102	Santa Cruz	North Coast	2000
103	Santa Cruz	North Coast	2000
104	Santa Cruz	North Coast	2000
201	Santa Fé	Central Barranco	2002
301	Isabela	Villamil and El Lagoon	2000
301	Isabela	Villamil and El Lagoon	2002
302	Isabela	San Tomás	2002
304	Isabela	Caleta Iguana Punta Essex	2002
306	Isabela	Punta Cristóbal	2002
307	Isabela	Caleta Webb	2002
606	Fernandina	Los Túneles	2002
701	Pinzón	Crater Base	2002

Table 3.2.2 *Solanum galapagense* populations used in this analysis

Pop no.	Island	Pop name	Year
301	Isabela	Villamil and El Lagoon	2000
301	Isabela	Villamil and El Lagoon	2002
302	Isabela	San Tomás	2002
303	Isabela	San Pedro	2002
306	Isabela	Punta Cristóbal	2002
308	Isabela	Playa Tortuga Negra	2002
602	Fernandina	Cabo Douglas	2002
603	Fernandina	Cabo Hammond	2002
605	Fernandina	5k E Cape Douglas	2002
606	Fernandina	Los Túneles	2002
702	Pinzón	North Coast	2002
801	Santiago	Bartolomé	2000

Table 3.2.3 *Solanum pimpinellifolium* populations used in this population analysis

Pop no.	Island	Pop name	Year
109	Santa Cruz	Los Gemelos	2000
109	Santa Cruz	Los Gemelos	2002
110	Santa Cruz	El Chato	2000
110	Santa Cruz	El Chato	2002
111	Santa Cruz	Bella Vista	2000
112	Santa Cruz	Road to Garapaterra Beach	2002
113	Santa Cruz	Road to Garapaterra Beach	2002
114	Santa Cruz	Puerto Ayora	2000
114	Santa Cruz	Puerto Ayora	2002
115	Santa Cruz	Old Basura	2000

Table 3.2.4 *Solanum pimpinellifolium* populations used in this population analysis

Pop no.	Island	Pop name	Year
301 *	Isabela	Villamil and El Lagoon	2000
302 *	Isabela	San Tomás	2000
302 *	Isabela	San Tomás	2002

RESULTS

Twenty loci in 11 enzyme systems were stained and successfully analysed. Nine loci in six enzyme systems were found to be polymorphic in at least one individual each – (PGM-1, PGM-2, PGI-2, MDH-3, IDH-1, 6-PGD-1, DIA-1, DIA-2 and DIA-3). Eleven loci were found to be invariable in all populations (ACN-1, ACN-2, AAT-1, AAT-2, SKDH, MDH-1, MDH-2, TPI-1, TPI-2, 6-PGD-2 (UGPP-2 was found to be variable in a single seed bank accession see Chapter 3c).

Species-specific alleles were found in all four taxa in the wild collected tomatoes from the Galápagos Islands (see frequencies in Table 3.3). In addition to this the two endemic tomatoes shared two private alleles, at near fixation, indicating common descent.

The two endemic Galápagos tomatoes *S. cheesmaniae* and *S. galapagense* share a fixed private allele (PGM-2-113). *Solanum cheesmaniae* has private (not fixed) alleles at IDH-95 at the north coast of Isla Baltra and two populations from the north coast of Isla Santa Cruz). The allele DIA-3 132 is private in *S. cheesmaniae* from Mirador and Caleta Iguana on Isla Isabela and also in very

low frequency in *S.cheesmaniae* and *S. pimpinellifolium* (one plant in each) from the Baltra Road, Isla Santa Cruz). *Solanum galapagense* is fixed in one private allele (MDH-3 104) and has private alleles at PGI-2-130 at El Lagoon de Manzanilla on Isla Isabela) and PGI -2-145 (Islas Bartolomé and Rábida) and 6-PGD-103 (San Pedro, Isla Isabela) DIA -1- 85 (southern regions of Isabela and from Islas Bartolomé, Pinzón, Rábida). *Solanum pimpinellifolium* is fixed and private at DIA-2-100 and private for PGM-1 106. *Solanum lycopersicum* has a unique combination of alleles allowing it to be distinguished from the other taxa. It shares the DIA-2-108 with *S. cheesmaniae* and *S. galapagense* (while *S. pimpinellifolium* has DIA-2-100) and *S. lycopersicum* also shares the PGM-2-100 (while *S. cheesmaniae* and *S. galapagense* have the PGM-2-113).

Although only pure populations (defined by morphology in the field) were analysed here, results show that there are individual plants that are putative hybrids. Putative hybrids are found to involve all of the taxa - *S. cheesmaniae* x *S. galapagense, S. cheesmaniae* x *S. pimpinellifolium* and *S. galapagense* x *S. lycopersicum*.

Allele frequency and allele presence tables

Allele frequency tables were created for the SCD collections using the individual data set (*sensu* De Walt and Hamrick, 2004). Following this approach, a single individual offspring was randomly selected to represent each field-collected accessions (see in General Materials and Methods above for further details). Variable loci, alleles and allele frequencies for SCD wild collections are presented in Table 3.3 covering *S. cheesmaniae*, *S. galapagense*, *S. pimpinellifolium* and *S. lycopersicum.*

A table showing the presence of alleles is given in Table 3.4 to represent overall allelic diversity in the SCD bulk collections (*sensu* De Walt and Hamrick., 2004). Please note that drift occurred when we selected single individual offspring for the individual data set. Thus, some diversity that was discovered in the wild/bulk collections was not recovered in the randomly selected individual plants.

Table 3.3 Variable loci, alleles and allele frequencies in *S. cheesmaniae*, *S. galapagense*, *S. pimpinellifolium* and *S. lycopersicum.* (SCD collection 2000 and 2002) (continued overleaf).

Taxa		*S. cheesmaniae*													
		2000				2000	2000	2002					2000	2002	2002
Island		Santa Cruz				Baltra	Isabela						Pinzón	Fernand.	Santa Fé
Population name		North coast	North coast	North coast	North coast	North Coast	El Lagoon de Manzanialla	El Lagoon de Manzanilla	Mirador	Caleta Iguana	Punta Cristobal	5km NE C. Webb	Crater base	W of Los Tunales	Center of island
Pop. No.		101	102	103	104	100	301	301	302	304	306	307	701	606	201
Locus															
PGI-2	**n**	**9**	**16**	**2**	**3**	**2**	**5**	**24**	**3**	**6**	**6**	**11**	**13**	**8**	**2**
	160	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	145	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	130	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	121	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PGM-1	**n**	**7**	**15**	**2**	**3**	**2**	**5**	**24**	**3**	**6**	**6**	**11**	**13**	**8**	**2**
	106	.	.	.	.	.	.	.	.	.	.	.	0.04	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96	1.00	1.00
PGM-2	**n**	**7**	**15**	**2**	**3**	**2**	**5**	**24**	**3**	**6**	**6**	**11**	**13**	**8**	**2**
	129	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	113	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.	1.00	1.00
	100	.	.	.	.	.	.	.	.	.	.	.	1.00	.	.
MDH-3	**n**	**9**	**16**	**2**	**3**	**2**	**5**	**24**	**3**	**6**	**6**	**11**	**13**	**6**	**2**
	104	.	.	.	.	.	.	.	.	.	0.50	.	.	0.17	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	1.00	1.00	0.83	1.00
	79	.	.	.	.	.							.	.	.
IDH-1	**n**	**9**	**16**	**2**	**3**	**2**	**5**	**24**	**3**	**6**	**6**	**11**	**13**	**8**	**2**
	100	1.00	0.94	.	.	.	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	95		0.06	1.00	1.00	1.00	.	.	.	.	.	.	.	.	.
6-PGD-2	**n**	**9**	**16**	**2**	**3**	**2**	**5**	**24**	**3**	**6**	**6**	**11**	**13**	**8**	**2**
	103	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	82	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DIA-1	**n**	**9**	**16**	**2**	**3**	**2**	**4**	**24**	**3**	**6**	**6**	**11**	**13**	**8**	**2**
	110	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	85	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DIA-2	**n**	**9**	**16**	**2**	**3**	**2**	**5**	**24**	**3**	**6**	**6**	**11**	**13**	**8**	**2**
	115	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	108	0.94	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	100	0.06				.	.	.	.	.	.	.	.	.	.
DIA-3	**n**	**9**	**16**	**2**	**3**	**2**	**5**	**24**	**3**	**6**	**6**	**11**	**13**	**8**	**2**
	132	.	.	.	.	.	.	.	1.00	1.00	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.	.	1.00	1.00	1.00	1.00	1.00

Table 3.3 continued

Taxa		*S. galapagense*												
		2000	2002						2000	2002			2002	2000
Island		Isabela							Santiago	Fernadina			Pinzón	Rabída
Population name		El Lagoon de Manzanilla	El Lagoon de Manzanilla	Road to San Tomas	San Pedro	Punta Cristobal	Playa Tortuga	Between Vol. Ecuador/Wolf	Islote Bartolomé	Cabo Douglas, Los Tunales	Cabo Hammond	5k E Cabo Douglas	W of Los Tunales	North west cost	Northern tourist landing
Pop. No.		301	301	302	303	306	308	309	801	602	603	605	606	702	501
Locus															
PGI-2	**n**	**4**	**23**	**4**	**12**	**5**	**14**	**1**	**12**	**5**	**1**	**6**	**7**	**2**	**1**
	160	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	145	.	.	.	.	.	.	.	0.08	.	.	.	.	.	1.00
	130	.	0.52	.	.	.	.	.	.	.	.	.	.	.	.
	121	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	0.48	1.00	1.00	1.00	1.00	1.00	0.92	1.00	1.00	1.00	1.00	1.00	.
PGM-1	**n**	**4**	**23**	**4**	**12**	**5**	**14**	**1**	**11**	**5**	**1**	**6**	**7**	**2**	**1**
	106	.	.	.	.	.	.	.	.	.	.			.	.
	100	1.00		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PGM-2	**n**	**4**	**23**	**4**	**12**	**5**	**14**	**1**	**12**	**5**	**1**	**6**	**7**	**2**	**1**
	129	.	.	.	.	.	.	.	.	.	.			.	.
	113	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	100	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MDH-3	**n**	**4**	**23**	**4**	**12**	**5**	**14**	**1**	**12**	**5**	**1**	**6**	**7**	**2**	**1**
	104	1.00	0.96	1.00	1.00	1.00	1.00	1.00	1.00	0.80	1.00	1.00	1.00	1.00	1.00
	100	.	0.04	.	.	.	.	.	.	0.20	.	.	.	.	.
	79	.	.	.	.	.	.	.	.	.	.	.	.	.	.
IDH-1	**n**	**4**	**23**	**4**	**12**	**5**	**14**	**1**	**12**	**5**	**1**	**6**	**7**	**2**	**1**
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	95	.	.	.	.	.	.	.	.						.
6-PGD-2	**n**	**4**	**23**	**4**	**12**	**5**	**14**	**1**	**12**	**5**	**1**	**6**	**7**	**2**	**1**
	103	.	.	.	0.17	.	.	.	.	.	.			.	.
	100	1.00	1.00	1.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	82	.	.	.	.	.	.	.	.	.	.			.	.
DIA-1	**n**	**4**	**23**	**4**	**12**	**5**	**14**	**1**	**12**	**5**	**1**	**6**	**7**	**2**	**1**
	110	.	.	.	.	.	.	.							
	100	0.25	0.33	1.00	0.75	1.00	1.00	1.00		1.00	1.00	1.00	1.00	0.25	.
	85	0.75	0.67		0.25				1.00	.	.			0.75	1.00
DIA-2	**n**	**4**	**23**	**4**	**12**	**5**	**14**	**1**	**12**	**5**	**1**	**6**	**7**	**2**	**1**
	115	.	.	.	.	.	.	.							
	108	1.00	0.96	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	100	.	0.04	.	.	.	.	.	.						.
DIA-3	**n**	**4**	**23**	**4**	**12**	**5**	**14**	**1**	**12**	**4**	**1**	**6**	**4**	**2**	**1**
	132	.	.	.	.	.	.	.	.						.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 3.3 continued

Taxa		*S. pimpinellifolium*										*S. lycopersicum*		
		2002				2002						2000		2002
Island		Santa Cruz										Isabela		
Population name		Los Gemelos	El Chato	Puerto Ayora	Old Basura	Los Gemelos	El Chato	Bella Vista	Garapaterro Rd	Garapaterro Rd	Puerto Ayora	Villamil	San Tomás	San Tomás
Pop. No.		109	110	114	115	109	110	111	112	113	114	301	302	302
Locus														
PGI-2	**n**	**9**	**22**	**5**	**1**	**3**	**2**	**1**	**6**	**1**	**1**	**2**	**5**	**3**
	160	.	.	.	.	.	.	.	.	.	.	.	.	.
	145	.	.	.	.	.	.	.	.	.	.	.	.	.
	130	.	.	.	.	.	.	.	.	.	.	.	.	.
	121	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
PGM-1	**n**	**7**	**25**	**6**	**1**	**3**	**2**	**1**	**6**	**1**	**1**	**2**	**4**	**3**
	106	0.86	0.96	0.42	1.00	.	1.00	.	0.17	1.00	1.00	.	.	.
	100	0.14	0.04	0.58	.	1.00	.	1.00	0.83	.	.	1.00	1.00	1.00
PGM-2	**n**	**8**	**22**	**5**	**1**	**3**	**2**	**1**	**6**	**1**	**1**	**2**	**5**	**3**
	129	.	.	.	.	.	.	.	.	.	.	.	.	.
	113	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MDH-3	**n**	**9**	**25**	**6**	**1**	**3**	**2**	**1**	**6**	**1**	**1**	**2**	**5**	**3**
	104	.	.	.	.	.	.	.	.	.	.	.	.	1.00
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.
	79	.	.	.	.	.	.	.	.	.	.	.	.	.
IDH-1	**n**	**8**	**22**	**5**	**1**	**3**	**2**	**1**	**6**	**1**	**1**	**2**	**5**	**3**
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	95	.	.	.	.	.	.	.	.	.	.	.	.	.
6-PGD-2	**n**	**9**	**25**	**6**	**1**	**3**	**2**	**1**	**6**	**1**	**1**	**2**	**5**	**3**
	103	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	82	.	.	.	.	.	.	.	.	.	.	.	.	.
DIA-1	**n**	**9**	**25**	**6**	**1**	**3**	**2**	**1**	**6**	**1**	**1**	**2**	**5**	**3**
	110	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	85	.	.	.	.	.	.	.	.	.	.	.	.	.
DIA-2	**n**	**9**	**25**	**6**	**1**	**3**	**1**	**1**	**4**	**1**	**1**	**2**	**5**	**3**
	115	.	.	.	.	.	.	.	.	.	.	.	.	.
	108	.	0.02	.	.	.	.	.	.	.	.	1.00	1.00	1.00
	100	1.00	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.	.	.
DIA-3	**n**	**9**	**25**	**6**	**1**	**3**	**2**	**1**	**6**	**1**	**1**	**2**	**5**	**3**
	132	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Legend

	Allele private to *S. cheesmaniae*
	Allele private to *S. galapagense*
	Alleles private to *S. cheesmaniae* and *S. galapagense*
	Alleles private to *S. cheesmaniae*, *S. galapagense* and *S. lycopersicum*
	Alleles private to *S. pimpinellifolium* and *S. lycopersicum*
	Alleles private to *S. pimpinellifolium*
	Indications of *S. cheesmaniae* x *S. galapagense* hybridization
	Indications of *S. cheesmaniae* x *S. pimpinellifolium* hybridization
	Indications of hybrization with other taxa

Table 3.4 Allele presence SCD bulk data set.

	allele	*S. cheesmaniae*							*S. galapagense*						*S. pimpinellifolium*		*S. lycopersicum*
		Baltra	Santa Cruz	Santa Fé	Isabela	Fernandina	Pinzón		Isabela	Rabida	Fernandina	Pinzón	Santiago		Santa Cruz		Isabela
PGI -2	160																
	145																
	130																
	121																
	100																
UGPP-2	112																
	100																
PGM-1	106																
	100																
PGM-2	129																
	113																
	100																
MDH-3	104																
	100																
	79																
6-PGD-2	114																
	103																
	100																
IDH-1	100																
	95																
DIA-1	110																
	100																
	85																
DIA-2	115																
	108																
	100																
DIA-3	132																
	100																

Legend

	S. cheesmaniae
	S. galapagense
	S. pimpinellifolium
	S. lycopersicum

Within population genetic diversity measures

The within population genetic diversity was calculated via the programme POPGENE. Table 3.5 shows a summary of the within population genetic diversity measures for the pure taxa. The overall genetic diversity is extremely low at just over one allele per locus for all taxonomic groups except *S. lycopersicum*, which has exactly one allele per locus. This shows that the mean *Ne* (estimated effective number of alleles) for the two introduced taxa is lower than that of the endemic taxa.

Furthermore, N_a (actual alleles) for all taxa is very low and N_e even lower showing that the alleles are not evenly distributed and are not equally contributing to the overall diversity, as some alleles were very rare. The N_a for *S. cheesmaniae* and *S. galapagense* are very low at 1.0179 and 1.0357 respectively. This shows that heterozygosity is rare. N_e is even lower 1.0061 and 1.0171 respectively. While N_e is very low for all groups, within the pure taxa *S. galapagense* was found to have the highest N_e, followed by *S. pimpinellifolium, S. cheesmaniae* and *S. lycopersicum.*

Figure 3.1. shows the within population genetic diversity measures for the two endemic taxa displayed east to west for the different islands, to establish if there is a geographic cline between the youngest and oldest islands. Figure 3.1.1 for *S. cheesmaniae* shows that there is a cline but this could be an artifact due to hybridization and other factors. No geographic pattern was detected for *S. galapagense* (Figure 3.1.2). It should be noted that Isla Pinzón was only represented by two plants - one of which was heterozygote at one locus while Isla Isabela was represented by 63 plants within seven populations. Plants on Isla Fernandina had the lowest N_e.

F-statistics

The summary of the F-statistics results are given in Table 3.6. and will be discussed later.

Neutrality tests

Neutrality tests were undertaken on the SCD collection. The mean for each locus is well within the upper and lower 95% range demonstrating that there is no evidence of selection in any of the loci tested for the SCD collections. Therefore, the allozyme loci investigated here were deemed to be selectively neutral.

STRUCTURE results

The likelihood graph for each cluster number is shown in Figure 3.2. K=3 was found to be the most likely scenario, the likelihood score plateaued after this k value. The STRUCTURE assignment bar charts are shown below (Fig. 3.3). The value of K=3 was also congruent with the morphological species circumscription (Fig. 3.3.2) as the three taxa separated along the species delimitations established with morphology. There were a few individual plants that were exceptions in all taxa. Some of these exceptions were plants that showed evidence of hybridization and were sympatric with other taxa – for example in the case of El Lagoon de Manzanilla (Isla Isabela). The data from K=2 to K=4 showed that there was a population within the *S. cheesmaniae* (defined using morphology) that did not conform to the usual alleleic structure for this taxa. The whole of the *S. cheesmaniae* population on Isla Pinzón corresponds to this unusual alleleic pattern and the individuals appear to be more closely aligned with *S. pimpinellifolium*. Although plants from the Isla Pinzón population were morphologically very consistent with other individuals belonging to *S. cheesmaniae* the STRUCTURE results (and the frequency tables) indicate that these plants are most likely to be of hybrid origin and the most likely species that they have formed hybrids with is *S. pimpinellifolium*.

Hybrids

Some evidence of hybridization was found between several of the taxa see the frequency table (Table 3.3) and the presence table (Table 3.4) and STRUCTURE results Fig. 3.3). Evidence of hybridization between *S. cheesmaniae* and *S. galapagense* was found at Los Túnales on Isla Fernandina and at Punta Cristóbal on Isla Isabela. Evidence of introgression in *S. cheesmaniae* from *S.pimpinellifolium* was found on Isla Pinzón. Some

populations of *S.pimpinellifolium* were found to have some alleles more usually found in *S. cheesmaniae* and *S. lycopersicum*. There was some evidence of alleles from *S. galapagense* in *S. lycopersicum* plants at San Tomás on Isla Isabela.

Table 3.5 Summary of within population genetic diversity for all pure taxa within the Galápagos Islands collected by SCD**.**

Note

Na (obs. no. alleles Kimura and Crow 1964)

Ne (effective no alleles)

I (shannon's information index (Lewontin 1972)

H_O (Observed heterozygosity Levene 1949)

H_e (Expected heterozygosity Levene 1949)

Nei (1976) expected heterozygosity

Pop name	No. isles	No. pop.	n.	Year	N_a	N_e	I	H_o	H_e	Nei
S. cheesmaniae	6	14	106	2000/02	1.0179	1.0061	0.0063	0.0019	0.0046	0.0038
S. galapagense	5	9	98	2000/02	1.0357	1.0171	0.0164	0.004	0.0116	0.0106
S. pimpinellifolium	1	14	55	2000/02	1.0278	1.0102	0.0101	0.001	0.0068	0.0063
S. lycopersicum	1	3	10	2000/02	1	1	0	0	0	0
S. pimpinellifolium **Basura (2000)**	1	1	10	2000	1.05	1.0441	0.0331	0.0125	0.025	0.0234

Table 3.6 Summary of the F-statistics 'pure' *S. cheesmaniae*, *S. galapagense*, *S. pimpinellifolium* and *S. lycopersicum* (SCD individual).

Test group		**Mean**			
	N pops	**N ind**	**F_{is}**	**F_{it}**	**F_{st}**
S. cheesmaniae Mean	14	7.6	0.3514	0.9493	0.9218
S. galapagense Mean	14	7	0.6651	0.8952	0.6871
Endemic tomato mean	/	/	0.5804	0.9443	0.8673
S. pimpinellifolium	10	5.5	0.7022	0.9197	0.7303
S. lycopersicum	3	3.3	/	/	/

Figure 3.1.1 N_e arranged from east to west for *S. cheesmaniae*

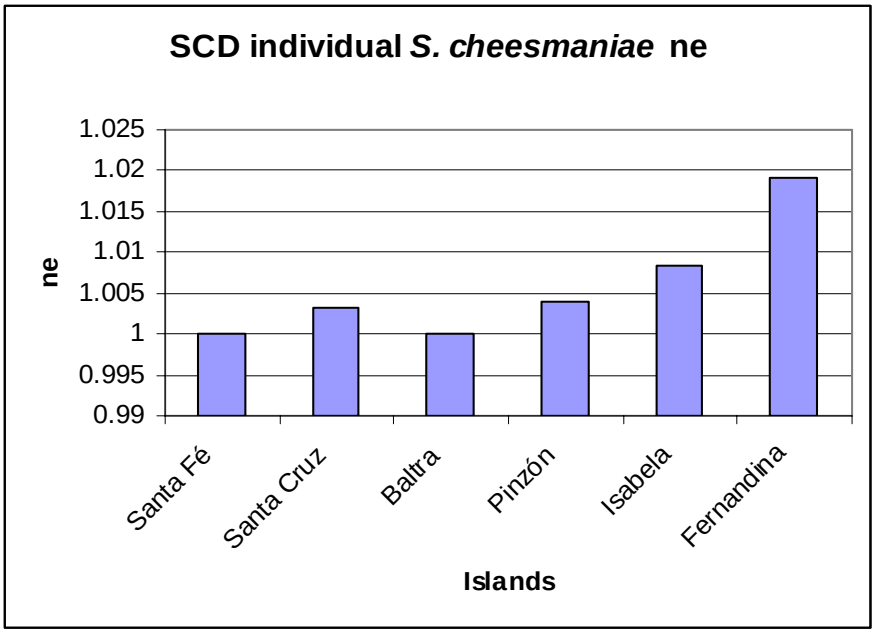


Fig 3.1.2 N_e arranged from East to West for *S. galapagense*

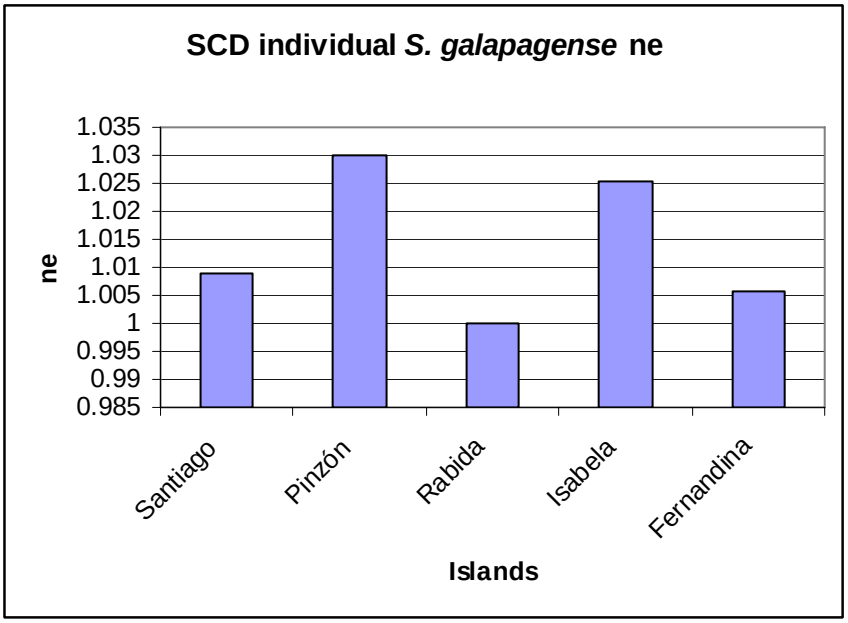


Figure 3.2 Plot of likelihood value of different values of k

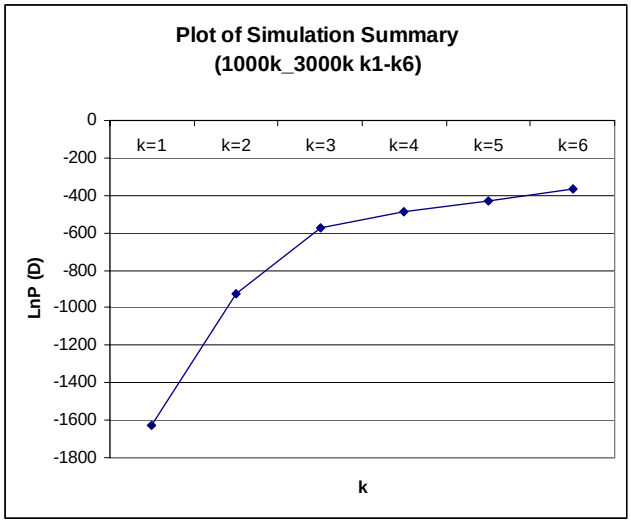


Figure 3.3.1 STRUCTURE bar chart (1000k_3000k_k=2)

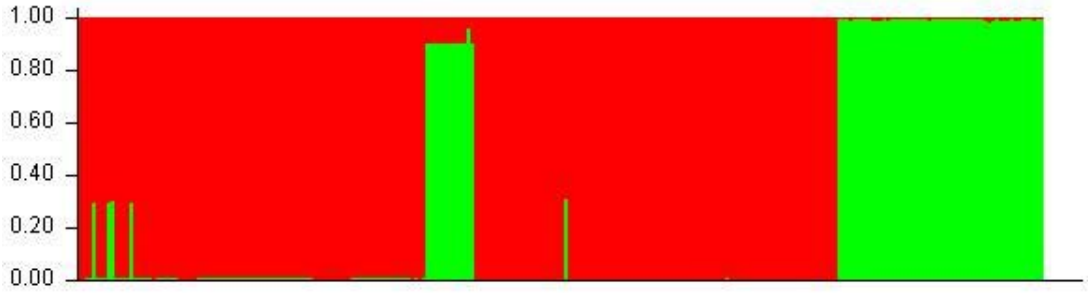
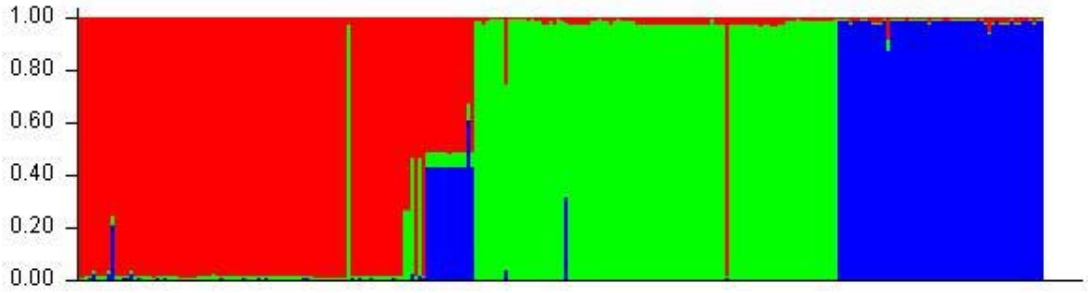


Figure 3.3.2 STRUCTURE bar chart (1000k_3000k_k=3)



S. cheesmaniae | ***S. galapagense*** | ***S. pimpinellifolium***

Figure 3.3.3 STRUCTURE bar chart (1000k_3000k_k=4)

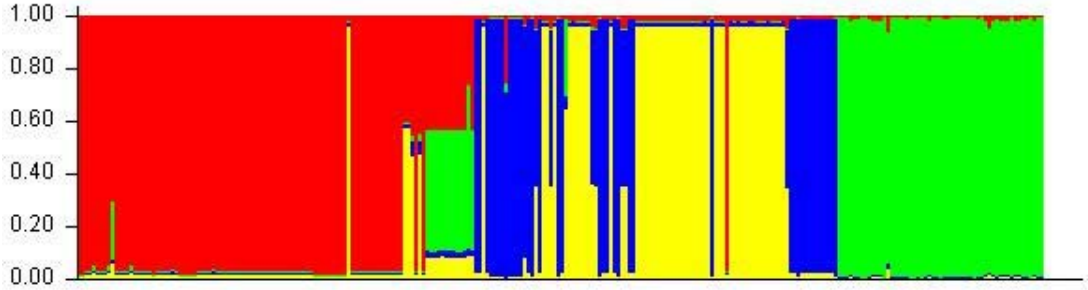


Figure 3.3.4 STRUCTURE bar chart (1000k_3000k_k=5)

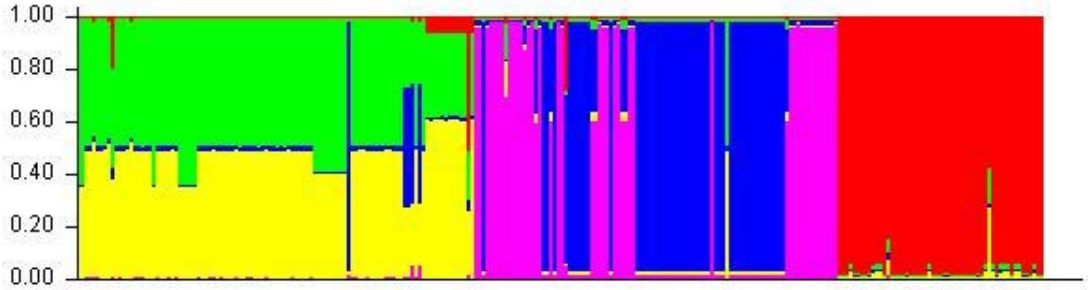
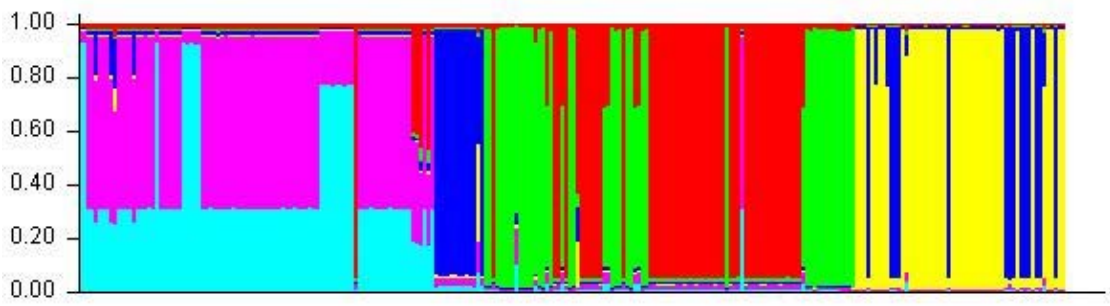


Figure 3.3.5 STRUCTURE bar chart (1000k_3000k_k=6)



Discussion

In summary, the main conclusions from the genetic investigation are:
1) Fixed, private or unique combinations of alleles were found in all four of the tomato taxa from the Galápagos Islands. Thus all four taxa can consistently be discriminated by allozyme electrophoresis. This is congruent with the morphological taxonomic treatment outlined in Chapter 2 (and Darwin *et al*., 2003),

2) *Solanum cheesmaniae* and *S. galapagense* shared one private allele at near fixation which indicates a shared evolutionary history.

3) *Solanum galapagense* had a distinct genetic identity with a private and fixed allele and two other private alleles at low frequency. This clearly separated *S. galapagense* from the other three taxa.

4) *Solanum pimpinellifolium* had a distinct genetic identity with one private and fixed allele and a second private allele. This clearly differentiated *S. pimpinellifolium* from the other three taxa

5) *Solanum lycopersicum* had a unique combination of alleles that distinguished it from the other species using these genetic markers although no private alleles were found in this species using these genetic markers.

6) Galápagos tomatoes as a whole had low levels of allelic variation and heterozygosity. More variation was found between populations than within

populations. *Solanum galapagense* was the most genetically variable out of the four tomato species growing in the Galápagos Islands.

7) Evidence of gene flow and hybridization was found in all groups of tomatoes. This was remarkable given the fact that the original genetic analysis was carried out on samples that had, in most cases, been field identified as morphologically pure taxa. However as stated in *Materials and Methods* the plants that were studied using allozyme electrophoresis were the offspring of the field collected plants so natural cross-pollination could have taken place in the field. Taxa that had been identified as putative hybrids will be analysed separately below.

Discussion of allelic diversity

Very low levels of outcrossing were found in the Galápagos tomatoes in general and this can be attributed in part to the low levels of bee activity in some areas and to flower morphology. Galápagos tomatoes are described as being highly autogamous and “Little or no activity of insect pollen vectors was observed under natural conditions” (Rick and Fobes, 1975). Being autogamous was probably essential for the ancestral form of the Galápagos tomatoes due to the paucity of pollinators in the islands (Rick and Fobes, 1975).

There was little genetic differentiation between the taxa and this was consistent with other studies, for example Rodrigez *et al*. (2009). However the difference that was found here was consistent, highly diagnostic and thus allowed the species to be clearly differentiated. The private and fixed alleles in two loci, DIA and PGM, found in the two endemic Galápagos tomato species indicated three things: 1) that they share a common ancestor (as was suggested by Rick and Fobes, 1975), 2) they are more closely related to each other than they are to either of the two introduced tomatoes and 3) that they have evolved in-situ from a common ancestor since their original colonisation. Charles Rick from the TGRC spent many years studying the Galápagos tomatoes and concluded that they had evolved monophyletically (Rick, 1975) and confirmed by Rodrigez *et al.* (2009).

Rick's (1975) allozyme study showed that the endemic Galápagos tomatoes were very uniform. Rick's opinion was that there was only one species of tomato endemic to the Galápagos Islands and he maintained the view throughout his research career. Rick identified several different types formally describing two distinct forms – *Lycopersicum cheesmanii* forma *typicum* and *Lycopersicum cheesmanii* forma *minor* (Rick, 1975). His taxonomic delimitation mirrored the species delimitation that I concluded – taxa now described as *S. cheesmaniae* and *S. galapagense* respectively. My study revealed a much clearer differentiation through fixed genetic variation between the endemic taxa than recovered by Rick and Fobes (1975), who used different enzyme systems.

Rick and Fobes (1975) and Nuez *et al.* (2004) found that *S. galapagense* (as *L. cheesmanii* forma *minor*) had the lowest allelic diversity despite it being the most widely distributed of the two endemic taxa. This was not consistent with my findings. Out of the two endemic Galápagos tomatoes, *S. galapagense* was found to have the highest diversity. Nuez *et al*. (2004) suggest that *S. galapagense* is the more recently evolved of the two Galápagos tomato species.

Rick (1975) stressed that of all Galápagos taxa, *S. galapagense* (as *L. cheesmanii forma minor*) was not only the most distinct taxon morphologically but also the most distinct allozymatically. My data are congruent with this and support this earlier assessment. Rick also found that *L. cheesmanii* (presumably including both forms) was closest allozymatically to *S. pimpinellifolium*. When studying morphology, *S.cheesmaniae* is most similar to *S. pimpinellifolium* of the two endemic taxa and it is thought that *S. pimpinellifolium* (or a taxon closely related to it) is the ancestor to the two endemic Galápagos tomatoes. However, sequencing of suitable genetic markers would need to be undertaken to verify this hypothesis.

Overall low levels of heterozygosity were found amongst the tomatoes in the Galápagos. Autogamous species have higher levels of homozygosity, lower levels of variation within population but usually higher levels of variation between populations due to genetic drift, whereas species that outcross usually have higher within population variation and less variation between populations,

(De Walt and Hamrick, 2004). All of this is mirrored in the observations of genetic diversity in the endemic Galápagos tomatoes and thus would indicate that the taxa are mostly inbreeding.

Geographic differentiation

Several alleles were found to be fixed or near fixation in several species. A good example is genetic variation in MDH-3. *Solanum galapagense,* for example, was fixed for the MDH-3 104 allele in with a few exceptions. In *S. cheesemaniae* MDH-3-104 was present in two populations, one at Punta Cristóbal Isla Isabela (pop. 306 –) and one at Los Túneles Isla Fernandina (pop 602 –). Conversely the MDH-3 100 allele, for which *S. cheesemaniae* is near fixation, was found in *S. galapagense* in Los Túneles Isla Fernandina (pop 602) and at El Lagoon de Manzanilla Isla Isabela (pop – 301).

On Isla Fernandina both taxa were found growing in sympatry at Los Túneles (pop 606). Evidence of hybridization between the species was recovered using allozymes, although no morphological evidence was apparent in the field or in greenhouse reared plants.

One interesting geographical pattern found in *S. galapagense* is DIA-1 85 allele (private to *S. galapagense*). This is found in the wild collected populations on the southern coast of Isla Isabela - in high frequencies at Villamil and San Pedro and also on Islas Pinzón, Bartolomé and Rabída. It is completely absent from SCD wild collections from Isla Fernandina and the west coast of Isla Isabela (but see seed bank Chapter 3c for comparison). This must be as a result of long distance dispersal.

The allele PGI-130 was private to *S. galapagense* and only in one population El Lagoon de Manzanilla on Isla Isabela. It was found in just over half the *S. galapagense* accessions collected here in 2002. However this allele was not sampled at all two years before, i.e. in 2000 field season. This may be an indication of high population turnover and drift or insufficient sampling size due to the low number of plants accessible in the wild.

Very few populations were sampled from the western side of Isabela and only one population was collected from the west of Isla Fernandina during this study. Rick and Fobes (1975) found that the allozyme diversity in the Galápagos tomatoes was higher on the western islands and particularly on their western slopes (although sample sizes were too small to show statistically significance). They speculated that this diversity might be due to the higher rainfall found on the western slopes of these islands. It could also be that the plants recolonise from several localities after volcanic activity creating a zone of high diversity through admixture as shown for several taxa in European phylogeographic studies, e.g. in beech (Comps *et. al*. 2001).

Hybrids

The analysis in this section only included individual plants and populations that were found to be morphologically 'pure' and could unequivocally be assigned to a specific taxon in the field. Chapter 3b covers the plants that were collected in the field and identified as being morphologically intermediate between different taxa. However, a brief discussion of hybrids is required here as some evidence of hybridization and gene flower was detected in what was assumed to have been 'pure' populations.

Before going into further details, I will reiterate that morphological identification of the taxa in the field is in most cases clear and unequivocal, but there was some evidence from allozyme that there are some *S. cheesmaniae* x *S. galapagense* hybrids in several populations. As hinted at above, both *S. cheesmaniae* and *S. galapagense* had alleles that are assigned as species specific at Los Túneles (Fernandina). At Punta Cristóbal (Isabela) *S. cheesmaniae* had an allele only found in *S. galapagense*. At both sites, both taxa grew close to each other. The TGRC collections also show sympatry for example *S. cheesmaniae* (LA1402) and *S. galapagense* (LA1403) on Isla Fernandina "West of Punta Espinoza" were growing close to each other. This TGRC accession was almost certainly the same locality as the SCD Los Túneles. Los Túneles is a relatively new name for this area on Isla Fernandina and thus earlier collectors might have described it as 'being near Punta Espinoza'.

The *S. cheesmaniae* accessions from Mirador and Caleta Iguana (Isla Isabela) also had a rare allele. This allele was also found in the hybrid zone along the Baltra Road in *S. cheesmaniae* and *S.pimpinellifolium* populations. The *S. cheesmaniae* accessions from Mirador were very unusual morphologically with very pale yellow fruit that could almost be described as cream in colour. The foliage is more in keeping with the *S. cheesmaniae* "Academy Bay" as outlined in Chapter 2. This is an example disjunction.

There was evidence of *S. pimpinellifolium* alleles in a population of *S. cheesmaniae* on Isla Pinzón. Alleles commonly found in *S. pimpinellifolium* from two loci were found in this population. This again was not supported with morphological evidence. There is no record of extant *S. pimpinellifolium* on Isla Pinzón but there once was a research camp on the island, thus human introduction and subsequent extinction of *S. pimpinellifolium* cannot be ruled out. Other possibilities are 1) introduction of *S. pimpinellifolium* due to the tortoise reintroduction from the breeding programme on Isla Santa Cruz from tortoises that were repatriated to Isla Pinzón, 2) seed dispersal from Santa Cruz (which is close to Isla Pinzón) or 3 pollen transfer via the Carpenter bee *Xylocopa darwini*.

In addition to the extensive hybrid zone along the Baltra Road on Santa Cruz, which is discussed in detail in chapter 3b, there was a single *S. cheesmaniae* plant from the North Coast population (101) that had a *S. pimpinellifolium* allele in one locus. Although this was just a single plant it could indicate gene flow from the Baltra Road hybrid populations into this seemingly pure population along the coast.

On Santa Cruz Island there were several populations of *S. pimpinellifolium* that were morphologically 'pure' but had either alleles from *S. cheesmaniae* or *S. lycopersicum* at El Chato and Garapaterra Road respectively. Although *S. cheesmaniae* was not collected near El Chato during my fieldwork, *S. cheesmaniae* populations are within the ten-mile radius of a carpenter bees' potential flying range. In the fields along the Garapaterra road *S. lycopersicum* plants were growing nearby the feral plants of *S. pimpinellifolium*.

Finally an allele private to *S. galapagense* was found in three accessions of *S. lycopersicum* in San Tomás (Isabela) collected in 2002. This could be due to one of several reasons: 1) this represents gene flow from local wild *S. galapagense* into feral *S. lycopersicum*, 2) that these *S. lycopersicum* plants have been used in a crop breeding programme with *S. galapagense* and then subsequently introduced to the Galápagos or 3) that a local farmer has been undertaking crop breeding to improve local crops. The former hypothesis seems to be the most likely and the latter the most unlikely.

Population diversity measures

Only 45% of the loci investigated among all taxa of tomatoes found in Galápagos were found to be polymorphic. In *S. cheesmaniae* only 30% of the loci were polymorphic and 33% of this was probably due to hybridization with another taxa. In *S. galapagense* only 25% of the loci were polymorphic and of this 40% was probably due to hybridization with other taxa. Hamrick and Godt (1990) found that on average 50% of loci in plant species were polymorphic using allozyme electrophoresis. The endemic Galápagos tomatoes have levels of polymorphism below this level, especially if one discounts the diversity that I attribute to hybridization and introgression.

Within *S. galapagense* only 33% of all the populations had polymorphic loci (40% of this is probably due to hybridization). The population with the most polymorphic loci was El Lagoon with 15% polymorphic loci (n=23). In *S. cheesmaniae* only 38% of the populations had polymorphic loci (40% probably due to hybridization). Of the few populations that had polymorphic loci it was found that only a single locus was polymorphic in each. Most populations were represented by very small sample sizes (n=25 maximum) and a single plant represented some populations. This will contribute to the lack of diversity found in these Galápagos tomatoes and be amplified by inbreeding/selfing. Hamrick and Godt (1990) found on average plant populations had 34% polymorphic loci. If hybrids are taken into consideration the levels of percentages of polymorphic loci (*P*p) is higher in other taxa. Research into the endemic species of *Tolpis* (Asteraceae) from the Canary Islands (Crawford *et al*., 2006) showed a wide

range of (*P*p) from 21% to 78%. The Galápagos tomatoes are at the lower end of this spectrum that shows they may be low in polymorphic loci even by oceanic island standards. The highly dynamic environment of the Galápagos Islands (Islas Isabela and Fernandina having frequent volcanic eruptions) will favour drift and this, combined with exceedingly small populations and thus sample sizes, will almost certainly influence these results.

The results for both *S. cheesmaniae* and *S. galapagense* show that the N_e for Isla Isabela is most diverse out of all the islands. In *S. galapagense* although Pinzón had the highest N_e, it should be noted that two plants only represent this 'population' on Pinzón. One of these plants is heterozygote at one locus (see frequency table – Table 3.3), which did make a big difference given the small sample size. In *S. galapagense*, Isla Isabela has the second highest N_e and was more informative as this island represented over three times more accessions than any other island and yielded more populations than the other islands. In small populations even a low level of heterozygotes can make a big difference to the diversity measures so caution is advised.

The tomato flower is hermaphroditic; it has both male and female reproductive parts in the same flower. While it is known to predominately self-pollinate, outcrossing is possible. This would allow for the potential of hybridization between all the taxa under investigation. Self-pollination causes lack of genetic recombination that can lead to inbreeding depression. However self-pollination may be preferential in some circumstances. For example, with Galápagos tomatoes, the ancestral tomato that originally colonized the islands, most probably did so in the absence of pollinating bees therefore being self-compatible would have been essential. Plants that occur at the edge of their range in very low numbers are often self-pollinating (known as Baker's Law (Baker, 1967). Selfing also prevents the introduction of genes from different populations, which could disrupt beneficial adaptive characteristics within the population (Lowe *et al*, 2004). In the case of the Galápagos tomatoes, self-pollination due to inserted stigmas leads to species isolation and controls the level of hybridization between *S. cheesmaniae* and *S. galapagense* in sympatric populations and islands.

In *S. cheesmaniae* and *S. galapagense* the actual number of alleles (N_a) was very low indicating that most individuals were homozygotic. In addition to this, the existing diversity was not distributed evenly. N_e which estimates the effective number of alleles per locus was much lower than the N_a (the actual number of alleles per locus). This demonstrated that the alleles were far from equally contributing to the overall diversity. In other words the presence of a second allele in a locus was very rare. Of the two endemic taxa, *S. galapagense* had slightly higher diversity, (see frequency tables Tables 3.3) with a few alleles that were rarely occurring in a couple of populations and an additional allele that occurred regularly in various populations and is discussed below. This low N_e was particularly marked in *S. cheesmaniae* showing that the presence of a second allele at a locus is very rare and unequally distributed.

Diversity measures showed that there was very little genetic diversity in Galápagos tomatoes. The low levels of heterozygosity can be explained by selfing, high levels of inbreeding and scarcity pollinators. Most populations of the endemic taxa were found to have included stigmas (shorter than the anther cone) with only the occasional individual with an exserted stigma. Only two populations were found to have a higher level of exserted stigmas. Inserted stigmas are thought to reduce crossbreeding (Rick et al., 1978) (but see Georgiady & Lord, 2002). This, in combination with genetic drift, accounted for the lack of diversity within the populations and the fixation of diversity in some populations. These processes can also potentially lead to speciation.

Hamrick and Godt (1990, 1996) outline five different life history traits that they found contribute to levels of allozyme diversity within plant populations. These life history traits are as follows: breeding system, seed dispersal mechanism, life form, geographic range and taxonomic status. In line with the Hambrick and Godt (1996) terminologies Galápagos tomatoes life history traits are: selfing/ mixed mating, ingested/gravity seed dispersal, short lived perennial, endemic and dicot. Hamrick and Godt (1996) analyzed two trait combinations in order to establish which combinations were most important in contributing genetic diversity to populations. They analysed the results of data collected in other studies and found that that traits involving breeding system explained relatively high levels of the genetic variation (39%). The lowest level of N_e in this

category was selfing and mixed breeding system with animal seed dispersal. The categories that contributed to genetic diversity included - life form (28%), seed dispersal mechanism (25%) and geographic range (25%). All these traits in the Galápagos tomatoes would seem to promote genetic depauperacy – lower N_e (as Aes in Hamrick and Godt 1990) were found in dicots (compared to gymnosperm or monocot), mid levels of N_e were found for the seed dispersal mechanism of either gravity or ingested and the geographic range category of being endemic has a lower N_e plants that have a wider range according to Hamrick and Godt (1990, 1996). All these life history traits described by Hamrick and Godt (1990, 1996) would seem to contribute to the low levels of diversity in the Galápagos tomatoes. In the Galápagos the tomatoes as mainly inbreeding (augmented by some pollination by the only bee species in the Galápagos the endemic carpenter bee *Xylocopa darwini*) and selfing, having an endemic geographic range and gravity or ingested method of seed dispersal. All of these factors are likely to be very important towards contributing this overall lack of genetic variation within populations.

The bar charts see Figs. 3.1.1 and 3.1.2 that were generated using N_e to establish the presence of a geographic cline between the older east part of the archipelago and the younger west part of the archipelago gave different results for *S. cheesmaniae* and *S. galapagense*. The results for *S. cheesmaniae* showed that there was a very slight increase in diversity towards the west part of the archipelago with Isla Fernandina, the most western island, the most diverse, but only one population (n=8) was discovered and studied. However genetic evidence of hybridization with *S. galapagense* was found in this population (which was thought to be ‘pure’ from my field observations and morphological studies) and this Los Túneles population was sympatric with accessions of *S. galapagense*. The Isla Pinzón population had slightly higher diversity than Santa Cruz but *S. cheesmaniae* on the Isla Pinzón population may also be introgressed, this time with *S. pimpinellifolium* and this is discussed later. Thus, Isabela was by far the most diverse (albeit very low levels of diversity) and was represented by six populations and 51 accessions. The pattern of this increased diversity towards the west would seem to be an artifact and more due to hybridization and sample sizes.

No east/west pattern or cline was identified in *S. galapagense*. Isla Pinzón was identified as having the highest N_e but caution is needed here as the sample here is only n=2 and one of the two accessions was heterozygous at one locus. It could be argued that this therefore should have been left out. Isabela is then shown to have the highest level of diversity probably again due to the larger population numbers of seven with a sample size of n= 63.

Rick's (1975) finding of higher levels of diversity in the western parts of the western islands may have been due to – then - undetected gene flow between *S. cheesmaniae* and *S. galapagense* and, like results found here, due to higher numbers of populations. Alternatively the lower levels reported here compared to Rick (1975) could be due to local extinctions of alleles due to volcanic eruptions on both Islas Fernandina and Isabela or goat predation or Isla Isabela.

In *S. galapagense* the DIA -1 85 allele was in high frequency in the southern part of Isabela (and high in Pinzón but fixed in Bartolomé). Further variation was found in PGI-2 and 6-PGD. This diversity is probably not due to hybridization with other taxa as these alleles were not found in any other taxa and corroborating evidence comes from the fact that these alleles are also represented in seed bank accessions (see Chapter 3c for more details).

This very slight increased level of allelic diversity found in the Isla Isabela *S. galapagense* could be due to the number of populations, the size of the island (Isabela is well over double the size of any other island in Galápagos), the range of different habitats, the level of disturbance due to regular volcanic eruptions goat predation and due to the length of the island - the probable re-colonization from a wide selection of different islands – Isla Fernandina to the west, Isla Santiago, Rabída, Pinzón and Santa Cruz from the east and even Isla Floreana from the south (where the DIA-1-85 was also found in the seed bank accessions). The only pattern then that can be concluded is that Isabela is most allelicly diverse in the Galápagos tomatoes and particularly so in *S. galapagense*. Diversity in *S. cheesmaniae* on Isabela is attributed to either hybridization with *S. galapagense* or may also be attributed to the larger island effect described above. Further genetic studies using molecular markers (e.g.

AFLP, microsatellites or ISSR) might help to elucidate these questions. The recent complete eradication of goats on Isla Isabela should bode well for the tomato populations on this island and future genetic studies on tomatoes could be used as a model system to provide evidence for the positive effects of herbivore eradication projects on the native, endemic and threatened flora.

Amongst the two introduced taxa the N_e was highest in *S. pimpinellifolium*. No diversity was found within *S. lycopersicum*, probably reflecting the fact that the widely cultivated edible tomato has a low genetic basis due to selective breeding.
In all test groups there was a very low proportion of polymorphic loci with a mean of < 5%, the allelic richness was only just over 1 showing that very few loci had more than one (see Table 3.3). Even when a locus was polymorphic the mean shows that it was only just over one allele per locus. The mean H_e for all groups and species is much higher than the H_o indicating an extreme departure from Hardy Weinberg equilibrium, a deficit of heterozygotes and inbreeding/ selfing. Hardy-Weinberg Principle states that "in a large randomly breeding (diploid) population allele frequency will remain the same from generation to generation; assuming no unbalanced mutation, gene migration, selection or genetic.
(http://www.nbii.gov/portal/server.pt?open=512&objID=403&&PageID=574&mode=2&in_hi_userid=2&cached=true).

Several populations exhibited a strong deficit of heterozygotes. This can in some cases be explained by sampling error, especially in the cases where the populations were very small. However some populations have up to 25 individuals (e.g. El Lagoon de Manzanilla at Isla Isabela) and still lacked heterozygotes. The lack of heterozygotes in this population might be due to founder effect, as El Lagoon de Manzanilla is a man made gravel pit of recent origin the extract used to build the run way for the local airport.

F-Statistics
F-Statistic was used to elucidate the partitioning of diversity between the different test groups, the data are summarized in tables 3.5 and 3.6. The F-statistics show that there were high levels of inbreeding in all taxa in both

individual and bulk data sets. In some cases this inbreeding was nearly complete (Table 3.6). .

The inbreeding coefficient (F_{is}) was lower in *S. cheesmaniae* than in *S. galapagense* (Table 3.6). This indicated that there might be more outbreeding in *S. galapagense* than in *S. cheesmaniae*. This difference was particularly marked in the individual dataset. This demonstrated that some out-breeding was taking place. In the case of *S. cheesmaniae*, as already alluded to above, this is probably mostly due to hybridization – for example in the Islas Pinzón, Isabela and Fernandina populations.

The high F_{st} in both endemic species, *S. cheesmaniae* and *S. galapagense*, demonstrated that there is little genetic exchange between populations in either taxon. F_{st} results can be compared to Hamrick and Godt (1996) overview data. Although G_{ST} and F_{ST} statistical tests are not exactly the same both G_{ST} and $F_{ST} = (H_T-H_S)/H_T$ are comparable (Lowe *et al*., 2004). The Hamrick and Godt (1996) analysis (Table 3.7) showed that average Gst for inbreeding dicots are at a similar level to *S. galapagense* (Table 3.8). The F_{st} for *S. galapagense* was also very similar to Hamrick and Godt (1996) data for Solanaceae (Table 3.7).

Table 3.7 Results for some of the life history traits (Hamrick and Godt 1996)

Hamrick and Godt (1996) Life history trait	**H_e (as H_{es})**	**F_{st} (as G_{st})**
Outbreeding dicot	0.165	0.184
Inbreeding dicot	0.091	0.587
Solanaceae	0.094	0.426

Table 3.8 He and Fst of *S. cheesmania*e and S. galapagense compared with Hamrick and Godt (1996) results

SCD individual collection	**H_e**	**F_{st}**
S. cheesmaniae	0.0046	0.9140
S. galapagense	0.0116	0.667

Table 3.3 (the allele frequency tables) demonstrate that there is little allelic diversity as a whole but some alleles are restricted to individual populations and this can be attributed to founder effects or bottlenecks. Comparing the two endemic taxa F_{st} was much higher in *S. cheesmaniae* but this taxon had a slightly lower F_{is}, thus having raised levels of outcrossing (Table 3.6). .

Solanum pimpinellifolium and *S. lycopersicum*

In comparison with plants growing in their native range, introduced plants are expected to have a lower within-population and higher among-population genetic variation. Exceptions to this general rule occur when the introduced species populations are the result of multiple introductions, introductions from multiple locations of its native range or introductions from large founding populations (DeWalt and Hamrick, 2004). Low levels of genetic variation were recovered from *Solanum pimpinellifolium*. However, the evidence was inconclusive to determine if this variation was due to multiple introductions or came from heterozygous seeds from one introduction. A more detailed study of genetic diversity in *S. pimpinellifolium* would be desirable to compare the diversity in natural versus introduced populations and to pinpoint the origin(s) of the introductions into the Galápagos Islands.

All three populations of *S. lycopersicum* were genetically uniform but representing two multilocus genotypes. The genetic make-up of one population (San Tomás Isla Isabela – see Table 3.3) could indicate introgression from *S.*

galapagense but as this study is restricted to the Galápagos archipelago we do not know about the extent of genetic variation in this taxon, and thus do not know the origin of its genetic diversity.

Genetics and taxonomy of native species

The first taxonomic record of the Galápagos tomatoes was in Hooker's (1847) enumeration of the plants of the Galápagos. He placed the Galápagos tomatoes into three species. Riley (1925) was the first to proposed that the Galápagos tomatoes should be differentiated at the species level, later Müller (1940) suggested that the two recognisable entities should be subspecies of the same species. This was supported by Rick (1971). Rick's (1971) biological species concept was the main reason that he proposed a sub-specific level for the Galápagos tomatoes. He undertook extensive breeding experiments and found them to be completely intercompatible. It is now understood that high numbers of species are capable of cross breeding and that it is not a practical species concept to uphold. I prefer to think of species as being a morphological cluster (Mallet, 1995). One thing has remained consistent throughout all the different taxonomic treatments is that *S. galapagense* is different from the other endemic taxa on the Galápagos Islands (Peralta *et al*., 2008).

There are also practical reasons to have the two Galápagos tomatoes as separate species. Both Galápagos tomatoes species have been used in plant breeding programmes to introduce a series of useful characteristics into cultivated tomatoes. Plants with these characteristics have been collected from the wild are often confined to one of the two species and sometimes to a single population (see Darwin *et al*., 2003 and references therein). Therefore recognising these taxa as two separate entities avoids confusion between the taxa.

In Chapter 2 we also described two morphological variants found within *S. cheesmaniae* and called these *S. cheesmaniae* "typical" and *S. cheesmaniae* "Academy Bay morph" (and intermediates between the two). No allelic diversity has been found in *S. cheesmaniae* that turned out to be diagnostic for the discrimination of these different morphotypes, but more sophisticated marker systems such as microsatellites or ISSR might fruitfully employed here to test whether there is some speciation going on here. The *S. cheesmaniae*

“Academy Bay morph” appears not to be the result of hybridization with other taxa, e.g. *S. pimpinellifolium*. However one population that was defined as being a member of the *S. cheesmaniae* ”Academy Bay morph” was found to have an allele that was also found in one accession at the Basura Population in the Baltra Road hybrid zone on Isla Santa Cruz (Table 3.3). It would be of great interest to continue to study the fate of this intriguing morphological variety.

Flower morphology and pollination

In Chapter 2 we found that most populations of *S. cheesmaniae* and *S. galapagense* had individuals with flowers with included stigmas whereas *S. pimpinellifolium* styles were mainly exserted from the staminal column. This was consistent with my field observations in 2002. While included styles prevent cross-pollination, exserted styles would promote cross-pollination (in the presence of a pollinating bee *Xylocopa* spp., Rick, 1983). However the level of inbreeding seems consistent across all the species tested despite differences in flower morphology. This indicates that paucity of pollinators may be important here.

A detailed discussion of pollination in Galápagos tomatoes is included general discussion and conclusion section Chapter 3b.

History of the Galápagos tomatoes

The fact that *S. cheesmaniae* and *S. galapagense* share a private fixed allele (derived novelty) indicated that they may share a common history. In addition to this they also share a unique fruit colour not found in the other ‘red’ fruited tomatoes (Rick, 1971). The two species probably evolved from a common ancestor in the islands (Spooner *et al*., 1992, Rodriguez *et al.* 2009).

Chapter 3b

Galápagos tomato soup: hybridization between tomato species

Introduction

Elton (1958) in his classic book on plant and animal invasions describes the effect of some invasive species as “ecological explosions”. Elton was one of the first authors to recognise the irreversible effect that invasive non-native species can have on the natural world (Parker and Reichard, 1998). Extinction by hybridization has rarely been recognised but it is a serious threat to rare island endemics (Levin et al, 1996), furthermore few cases have been studied using modern molecular techniques (Rhymer and Simberloff, 1996). The first person to recognise the importance of infiltration of germplasm from one species into another was Ostenfeld (Anderson, 1949).

Oceanic islands are thought to be particularly susceptible to invasive plant species (Cronk and Fuller, 1995) and they are areas of great conservation concern. Almost 50 percent of the Galápagos flora is now composed of non-native taxa, of these, 2.5 percent are described as invasive (Mauchamp, 1997), but this is an underestimate, as detailed studies are sparse.

Ellstrand, *et al*. (1999) reviewed hybridization between crop plants and their wild relatives and with the enormous spread of agriculture can cite many examples where man’s activities lead to new contacts between crops and native relatives. They also cite one well researched example from the Galápagos Islands, where it has been suggested that the endemic Galápagos cotton (*Gossypium darwinii*) could be at risk of extinction due to hybridization with the introduced crop plant *Gossypium hirsutum* (Ellstrand, *et al*, 1999). Allozyme analysis suggests that *G. hirsutum* alleles in *G. darwinii* are derived from a transfer via *G. hirsutum* and introgressed *G. barbadense* (Wendel and Percy, 1990).

The occurrence of feral plants of *Solanum lycopersicum* on Santa Cruz Island, Galápagos has been documented since the 1950s (Rick, 1956) and later also of *S.pimpinellifolium* (Rick, in lit., 1998) The potential for hybridization between these taxa was established by Rick at the Tomato Genetic Resource Centre (TGRC) through artificial crossings between *S. cheesmaniae* and *S. lycopersicum* resulting in completely fertile offspring. Furthermore, the seeds of these F1 hybrids did not exhibit the low untreated germability exhibited by pure *S. cheesmaniae* (Rick, 1956). Thus, if hybrids occur in the wild and if the F1 hybrids would be fertile and their seeds have a higher germinability then the hybrids would have a selective advantage over its ancestral taxa and this could pose a serious threat to the genetic identity of the endemic species.

Studying the effects of invasives in oceanic islands is highly relevant to future studies of regions where habitat fragmentation is occurring now. The detailed study of island invasions, the meeting of crop plans and their wild relatives in these vulnerable and restricted spaces and the threat of hybridization, introgression and potentially genetic erosion on oceanic islands could act as a model for other studies at continental scales.

All four species of tomatoes found in the Galápagos are capable of cross breeding. Rick (1963) conducted compatibility tests in which he showed that there was complete compatibility to produce fertile hybrids within the two endemic tomato taxa and between the endemic taxa and *S. lycopersicum* and *S. pimpinellifolium.* MacArthur and Chiasson (1946) conducted extensive crossing experiments with tomatoes including *S. lycopersicum* and *S. pimpinellifolium* and concluded that if a species concept were to be based purely on genetic criteria these species would be described as subspecies due to their complete compatibility. Rick was of the opinion that the endemic tomato taxa in the islands formed just one species and formally described two forms within the species due their ability to cross breed. Rick described these two forms as *L. cheesmanii forma typicum* and *L. cheesmanii forma minor* and they correspond to my circumscription of *S. cheesmaniae* and *S. galapagense* respectively.

Both endemic Galápagos tomatoes species reliably possess yellow to orange fruit when ripe; no native biotype has bright red fruit. This was also the opinion of the late Charles M. Rick, who considered any red-fruited tomatoes in the Galápagos Islands to be introduced taxa - either '*Lycopersicum pimpinellifolium'* or '*L. esculentum* var. *cerasiforme'* (*Solanum pimpinellifolium* or *S. lycopersicum* respectively) (C. Rick, *in litt*, 1998;). Thus fruit colour is a useful character to determine taxa in the field and obtain first indications of species presence and the potential of hybridization.

The earliest unequivocal evidence of the occurrence of introduced tomato species on the Galápagos Islands are a herbarium specimen from 1932 of *S. lycopersicum* (B, CAS) and an accession held by the TGRC collected in 1985 for *S. pimpinellifolium* (Darwin *et al.* 2003). Although one of the specimens collected by Charles Darwin from the Galápagos was later described as '*L. pimpinellifolium*' by Hooker (1847) this does not match the Linnaean type specimen (LINN) and was not the same species as the wild *S. pimpinellifolium* found in mainland South America. This will be discussed later but see also Peralta *et al*. (2008), chapter 3a and Darwin *et al*. (2003) for further details.

The following investigation refers to hybrid populations or hybrid individuals only. Thus, it includes plants that were described as being morphologically intermediate between two taxa and includes all hybrids between introduced and the endemic taxa. In addition to these putatively hybrid populations and individuals 'pure' populations have been included in order to make a comparison with the hybrids. Individuals that were described as being pure in the field but that were subsequently found using allozyme electrophoresis to be putative hybrids are analysed and discussed in the earlier chapter 3a.

Rick (1971) found evidence of hybrids between the two endemic taxa and made notes about this at various localities in the islands. I have not found any reference made by Rick or in the wider literature of the hybridization or indeed the potential threat of hybridization of the Galápagos tomatoes with the introduced tomato plants before Tye (in *litt.* 1999). In this he mentioned the possibility of hybridization prompted by my concerns. Nuez *et al.* (2004) found

no evidence for hybridization between *S. cheesmaniae* and *S. pimpinellifolium* (as *L. cheesmaniae* and *L. esculentum* ‘Gal *cer*’*).*

Questions:

In this chapter I want to address the following questions:
1) What genetic or morphological evidence is there to support the hypothesis of hybridization between tomato species in the Galápagos Islands?

2) Do results from genetic markers and morphological analysis give congruent results and provide evidence of hybridization between the different species of tomatoes growing in the Galápagos Islands?

3) How do the morphometric delimitations of *S. cheesmaniae* and *S. pimpinellifolium* and their hybrids from Baltra Road on Isla Santa Cruz correspond to the results from allozyme electrophoresis?

4) What recommendations do these results suggest for the future conservation of Galápagos tomatoes?

Materials and methods

The morphological characters established by Darwin *et al*. (2003 – see Chapter 2) to delimit species were re-applied and augmented by personal observations during the 2002 field season. Allozyme electrophoresis analysis was undertaken on three different groups of hybrids involving different taxa. Within population diversity measures were calculated for the different populations and are presented in Tables 3.9.1 to 3.9.5 below.

Populations under investigation

Table 3.9.1. *Solanum cheesmaniae*, S*. galapagense* and *S. cheesmaniae* x *S. galapagense* hybrids

taxa	Pop no.	Island	Pop name	Year
S. cheesmaniae	301	Isabela	El Lagoon	2002
S. galapagense	301	Isabela	El Lagoon	2002
S. che x *S. gal*	301	Isabela	El Lagoon	2002

Table 3.9.2. *Solanum pimpinellifolium*, *S. lycopersicum* and *S. pimpinellifolium* x *S. lycopersicum* hybrids

taxa	Pop no.	Island	Pop name	Year
S. pimpinellifolium	110	Santa Cruz	El Chato	2000
S. lycopersicum	302	Isabela	San Tomás	2000
S. pimp x *S. lyc*	xx	San Cristóbal	Puerto Moreno Baquerizo	2000

Table 3.9.3. *Solanum cheesmaniae* for the *S. cheesmaniae* x *S. pimpinellifolium* hybrid pops of Baltra road

taxa	Pop no.	Island	Pop name	Year
S. cheesmaniae	301	Isabela	El Lagoon de Manzanilla	2002
S. cheesmaniae	101-104	Santa Cruz	North Coast populations	2000
S. cheesmaniae	105	Santa Cruz	Zone of birds	2002
S. cheesmaniae	106	Santa Cruz	Mina Negra	2002
S. cheesmaniae	107	Santa Cruz	Basura	2002

Table 3.9.4. *Solanum pimpinellifolium* for the *S. cheesmaniae* x *S. pimpinellifolium* hybrid pops of Baltra road

Taxa	Pop no.	Island	Pop name	Year
S. pimpinellifolium	107	Santa Cruz	Basura	2000
S. pimpinellifolium	107	Santa Cruz	Basura	2002
S. pimpinellifolium	108	Santa Cruz	Mina Roja	2000
S. pimpinellifolium	108	Santa Cruz	Mina Roja	2002
S. pimpinellifolium	109	Santa Cruz	Los Gemelos	2000
S. pimpinellifolium	109	Santa Cruz	Los Gemelos	2002
S. pimpinellifolium	110	Santa Cruz	El Chato	2000
S. pimpinellifolium	110	Santa Cruz	El Chato	2002
S. pimpinellifolium	110	Santa Cruz	El Chato	2002
S. pimpinellifolium	111	Santa Cruz	Bella Vista	2000
S. pimpinellifolium	112	Santa Cruz	Garapaterra Road	2002
S. pimpinellifolium	113	Santa Cruz	Garapaterra Road	2002
S. pimpinellifolium	114	Santa Cruz	Old Basura	2000
S. pimpinellifolium	115	Santa Cruz	Puerto Ayora	2000
S. pimpinellifolium	115	Santa Cruz	Puerto Ayora	2002

Table 3.9.5. *Solanum cheesmaniae* x *S. pimpinellifolium* hybrid pops of Baltra road

taxa	Pop no.	Island	Pop name	Year
S. che x *S. pimp*	105	Isabela	Zone of birds	200?
S. che x S. pimp	106	Santa Cruz	Mina Negra	2000
S. che x S. pimp	107	Santa Cruz	Basura	2002
S. che x S. pimp	108	Santa Cruz	Mina Roja	2002

STRUCTURE Analysis

STRUCTURE analysis was performed on the putatively hybridising populations from Baltra Road using the same methods outlined in the General Materials and methods above. Two analyses were undertaken:

1) Hybrid 1 STRUCTURE analysis (SCD hybrid pops – BULK data set, with all offspring from each accession (average 3)). Morphology and allozymes.
2) Hybrid 2 STRUCTURE analysis (data set of SCD hybrid pops with one individual randomly selected per accession. Allozymes only.

STRUCTURE analysis output gives an assignment to a specified number of groups (in this case taxa). An appropriate threshold was established in which

the two pure taxa could be defined. Burgarella *et al*. (2009) wrote that "In the STRUCTURE model, the posterior probability (*q*) describes the proportion of an individual genotype origination from each of the K categories". Finding the optimal threshold value (*Tq*) of the *q* is important in the classification of purebred species or individuals with hybrid origins (Burgarella *et al*., 2009). The *Tq* purity thresholds of 93% (Hybrid 2 analysis) and 85% (Hybrid 1 analysis) were used during the research into *S. cheesmaniae* x *S. pimpinellifolium* hybrids along the Baltra Road.

Hybrid 1 STRUCTURE analysis (Baltra Road, Isla Santa Cruz and El Lagoon de Manzanilla, Isla Isabela).

The purpose of this analysis was to make a comparison between the phenotypes of the Galápagos field collected accessions and the genotypes of the UK greenhouse grown offspring (i.e. the parental phenotype and the offspring genotype). of the following areas 1) *S. cheesmaniae* El Lagoon on Isla Isabela, 2) the Baltra Road *S. cheesmaniae*, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* hybrid populations 3) the *S. pimpinellifolium* El Chato populations.4) the *S. pimpinellifolium* Garapaterra Road populations See Table 3.10 for summary of taxa. This analysis was undertaken using the bulk data set (i.e., all offspring grown from individual accessions collected in the field. The burn-in period was 800,000 and iterations 2000,000 (800k, 2000k).

The El Lagoon de Manzanilla population (Isla Isabela) of *S. cheesmaniae* was used to represent the pure *S. cheesmaniae* as a comparison. These accessions were found to be the closest large population that was allozymatically identical to the pure *S. cheesmaniae* found along the Baltra road that was collected in 2002 (see Table 3.14.1-3 for allele frequency of hybrid populations).

Table 3.10. Population names, numbers and putative taxa for the Hybrid 1 STRUCTURE analysis. All populations are from Isla Santa Cruz unless specified otherwise. All populations were collected in 2002

Putative taxa (based on morphology)	**Pop.**	**Population name**
S. cheesmaniae and *S. che* x *S. pimp*	105	Zone of birds
S. cheesmaniae and *S. che* x *S. pimp*	106	Mina Negra
S. cheesmaniae, *S. pimpinellifolium* and *S. che* x *S. pimp*	107	Basura
S. cheesmaniae, *S. pimpinellifolium* and *S. che* x *S. pimp*	108	Mina Roja
S. pimpinellifolium	109	Los Gemelos
S. pimpinellifolium	110	El Chato
'Pure' *S. pimpinellifolium*	112	Garapaterra Rd. (1)
'Pure' *S. pimpinellifolium*	113	Garapaterra Rd. (2)
Pure *S. Cheesmaniae*	301	El Lagoon (Isabela)

The Garapaterra Road populations (112 and 113) of *S. pimpinellifolium* were selected to represent 'pure' *S. pimpinellifolium* that was also collected in 2002*. Solanum pimpinellifolium* was found to form hybrids with *S. lycopersicum* (as well as *S. cheesmaniae*). For this analysis accessions which were morphologically intermediate between *S. lycopersicum* x *S. pimpinellifolium* from the Garapaterra Road were removed in order to try to reduce the levels of complexity in this analysis. Only individual accessions of *S. pimpinellifolium* that were morphologically classified (in the field) as purebred were included. However during this genetic analysis it was found that three out of these seven individuals showed evidence of introgression, most likely with *S. lycopersicum* (as it was growing locally).

As already outlined above in this chapter, the allozyme electrophoresis was undertaken on leaf samples from greenhouse raised plants that were grown from wild collected seeds; these samples were therefore the offspring of the field collected plants. On average 3.54 (1.31) offspring plants were analysed per field accession for the Baltra Road hybrids and the Garapaterra road *S. pimpinellifolium*. Only one offspring was grown per accession in the El Lagoon population of *S. cheesmaniae* –these were found to be genetically uniform (see Table 3.14 for frequencies).

Analyses using the assignment generated from the Hybrid 1 STRUCTURE analysis (bulk data set)

The threshold *q* (*Tq*) for this analysis was set at 85%. This corresponded to the morphological understanding of the accessions and the frequency tables 3.14 generated directly from allozyme data. On a scale of 0-1, the pure *S. cheesmaniae* plants were at the lower end of the q. Accessions with STRUCTURE assignments of <0.15 were designated as 'pure' *S. cheesmaniae*, those of >0.85 were designated as pure *S. pimpinellifolium* and STRUCTURE assignments that fell between 0.15-0.85 were designated as *S. cheesmaniae* x *S. pimpinellifolium* hybrids. (e.g., El Lagoon purebred *S. cheesmaniae* had a value of 0.037) and *S. pimpinellifolium* at the upper end at 1 (e.g., Garapaterra Road pure *S. pimpinellifolium* had values of 0.927 and 0.966).

From the STRUCTURE assignment (*q*) a mean was calculated from the offspring plants for each accession. This mean was then taken to represent the genetic makeup of the field collected parent accession. Only one plant was grown per accession in *S. cheesmaniae* from El Lagoon de Manzanilla so that the means could not be calculated for this population, however between accessions the allelic variation was completely uniform in this population. A mean of these posterior probabilities (*q*) generated in STRUCTURE were then used in the PCO for a comparison between parental phenotype and offspring genotype.

Hybrid 2 STRUCTURE analysis (individual data set)

The purpose of this analysis was to investigate and display the genetic diversity of the following three areas on Isla Santa Cruz 1) *S. cheesmaniae* Isla Santa Cruz North coast populations, 2) the Baltra Road *S. cheesmaniae*, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* hybrid populations 3) and the *S. pimpinellifolium* El Chato populations (see Table 3.11). Plants with missing data were removed for this analysis as it allowed a stricter threshold to be used. Population 10502 was also removed from this analysis because after the removal of the individuals with missing data points from this population - it was only represented by a single plant.

The STRUCTURE programme options were arranged as follows (see above in STRUCTURE analysis 7 for further details) Admix_corr 400k2000k. A *Tq* of 93% was established to define a pure offspring. This meant that an assignment of 0 – 0.07 = *S. cheesmaniae*, 0.07 - 0.93 = *S. cheesmaniae* x *S. pimpinellifolium* and >0.93 = *S. pimpinellifolium*. The total number of accessions corresponding to each of these three categories for each population was then calculated and the total for each of the three groups used to generate pie charts (EXCEL) using percentages.

Table 3.11. Population names, numbers and putative taxa for the STRUCTURE analysis Hybrid 2. All populations are from Isla Santa Cruz, the final two digits on the population number give the collection year (00 = 2000)

Putative taxa (based on field morphology)	**Pop. No.**	**Population name**
S. cheesmaniae	10100	North Coast 1
S. cheesmaniae	10200	North Coast 2
S. cheesmaniae	10300	North Coast 3
S. cheesmaniae	10400	North Coast 4
S. cheesmaniae and *S. cheesmaniae* x *S. pimpinellifolium*	10602	Mina Negra
S. cheesmaniae, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium*	10702	Basura
S. pimpinellifolium and *S. cheesmaniae* x *S. pimpinellifolium*	10802	Mina Roja
S. pimpinellifolium	10902	Los Gemelos
S. pimpinellifolium	11000	El Chato

Comparison between parental phenotype and offspring genotype

Normal distribution tests and then Wilcoxon tests (PAST, Hammer *et al.* 2001) were undertaken on Baltra Road hybrid populations to test whether the field circumscription of the different taxa was the same as the results obtained from allozyme electrophoresis analysed with STRUCTURE. The three groups that were included were: 1) *S. cheesmaniae*, 2) *S. cheesmaniae* x *S. pimpinellifolium* hybrid 3) *S. pimpinellifolium*. The taxa were defined in three different ways: 1) the field circumscription of each taxa 2) the STRUCTURE assignment at the *Tq* threshold of 0.15 and 3) the STRUCTURE assignment *Tq* threshold of 0.07.

Morphological analysis of the Baltra Road hybrid populations

Morphological classification and measurements of all accessions were undertaken in the field by SCD for all plants collected in 2002 (SCD collection numbers SCD-438 to SCD-842). Morphological characters were measured and observations were made on individual plant accession leaves, inflorescences, flowers, fruit and seeds. These characters were selected from Chapter 2 and personal observations from the field in 2000 and 2002. All morphological characters were 'measured' on living plants either in the field or soon thereafter (within 24hours). This was with the exception of leaf samples; these were pressed between kitchen roll and transparent sticky backed plastic to prevent shrinkage during the drying process. This method allows measurements to be made at a later date (method from I. Peralta pers. comm. 2002). All morphological measurements were undertaken by SCD to maintain continuity and to reduce measurement error.

For the morphological analysis of the Baltra Road hybrid populations of *S. cheesmaniae*, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* see Table 3.12 for summary of characters used in descriptive statistics, ANOVA TUKEY and PCO (both undertaken in PAST). Table 3.12 also gives a description of the characters. Table 3.13 outlines the methods used to score the fruit colour and figure 3.5 shows the methods used to score the sepal angle character. The characters are continuous, continuous discrete and ordinal (Table 3.19).

A selection of 17 characters were analysed using STATISTICA 6 (StatSoft Inc., 2001) to calculate means and 95% confidence intervals for both taxa and putative hybrids. These were represented in mean plots also generated in STATISTICA.

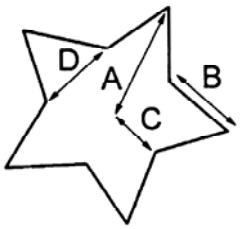
Morphological Characters

Table 3.12. Summary of morphological characters

code	Morphological character	Description
1	Flower A	Length from centre of the corolla to apex of the corolla lobe (mm)
2	Flower B	Length of the corolla lobe from apex to the corolla lobe junction (mm)
3	Flower C	Length from the centre of the corolla to the corolla lobe junction (mm)
4	Flower D	Corolla lobe width (mm)
5	Anther length	Overall length of anther (mm)
6	Stigma exsertion length	Length of the protrusion of the stigma from the end of the staminal column (mm)
7	Sepal length on flower	Length of sepal from flower base to tip (mm)
8	Sepal width on flower	Sepal width measured at widest point from same sepal as above (mm)
9	Inflorescence length	Length measured from the base of the basal flower to the base of the terminal flower petiole (mm)
10	Flower number per inflorescence	Count of the number of flowers per inflorescence from inflorescence measured above
11	Fruit size	Width at widest (mm)
12	Fruit skin thickness	Measured on fruit that has been cut open - the measurement between the interior of the fruit and the fruit skin (mm)
13	Fruit colour	Fruit colour assignment to a series of colours on a chart further details Outlined below
14	Seed number per fruit	Seeds counted per fruit measured above
15	Sepal length on fruit	Sepal length measured from attachment with the petiole on underside to the tip of the sepal (mm)
16	Sepal width on fruit	Sepal width measured from widest point from same sepal as above (mm)
17	Sepal angle on fruit	One of five sepal angles was assigned (further details outlined below)

Flower measurements

Fig. 3.4 Flower measurements



A: Length from centre of the corolla to apex of the corolla lobe
B: Length of the corolla lobe from apex to the corolla lobe junction
C: Length from the centre of the corolla to the corolla lobe junction
D: Corolla lobe width

Descriptive statistics and box plots were undertaken in STATISTICA on the Baltra Road *S. cheesmaniae*, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* (Santa Cruz pops, 106, 107 and 108) and *S. cheesmaniae* from El Lagoon de Manzanilla (Isabela pop 301) and *S. pimpinellifolium* from Garapaterra Road (Santa Cruz pops. 112 and 113). Two different groupings were analysed. First the accessions were divided into five groups based on locality and taxonomy – the three taxa from Baltra Road, one from El Lagoon and one from Garapaterra Road. Secondly, the accessions were divided on the basis of taxonomy – three groups – *S. cheesmaniae*, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* thus the pure taxa population of *S. cheesmaniae* and *S. pimpinellifolium* were merged with the *S. cheesmaniae* and *S. pimpinellifolium* from within the hybrid populations. The box plots are included in Appendix 1.

Fruit colour

Fruit colour was measured by matching the ripe fruit colour to one of 26 different colour cards (manufactured by Dulux^{TM}) in the field. These were then categorised into five different colour groups, which were ordered according to Newton's colour wheel. The discovery of the colour wheel is attributed to Sir

Isaac Newton who, using a light prism, interpreted the colours as a continuous 'wheel'. The colour wheel is therefore based on reality and not simply an arbitrary way of ordering and displaying colour.

Colour can be described in terms of hue, saturation and brightness. Hue is the wavelength of the spectrum. Saturation is the intensity of the colour. Brightness depends on luminance and reflectivity of the surface (http://hyperphysics.phy-astr.gsu.edu/HBASE/vision/colchar.html). Hue is the only component that was measured during this research to describe the different fruit colours of the tomatoes in Galápagos.

Tomatoes from the Galápagos Islands have fruit that are yellow, orange or red. Yellow and red are primary colours and orange - a secondary colour - formed by a mixture of yellow and red. The fruit colour is represented here in the order found on the colour spectrum/wheel (counter clockwise) - Yellow is scored -1, yellow/orange - 2, orange -3, red/orange - 4 and red - 5. For exact representation of the colour matched in the field using the Dulux colour code found in Table 3.13 (http://www.dulux.co.uk/colours/index.jsp). These Dulux codes have been scanned using a spectrophotometer (P. Hurst *in litt*.). There are two colour readings presented below - L*a*b* colour space reading and RBG (red/blue/green). These colours are available to be viewed on the internet using a colour metric converter (e.g. see www.colorpro.com/info/tools/convert.htm).

I endeavoured to make the description of the tomato fruit colour objective by matching the colours in the field to colour cards and scoring the colour in a continuous manner according to spectral hues. In the past colour was often scored in a more subjective way where the observer named the colour without reference. If colour characters were to be used again in research such as this, a spectrophotometer could be used directly on the fruit (for example see Bray *et al*., 2006). In the case of the RBG system the assignment to each of these 'primary' colours (in this case red, blue and green) could then be plotted on xyz axes respectively.

Table 3.13. SCD codes used to specify Dulux colours with corresponding readings in L*a*b* colour space and RBG

Yellow 1

SCD code	Dulux code	L*a*b* (lab colour space readings)			RBG		
		L	a	b	R	B	G
G14	60YY 64/526	83.2	-4.2	54.7	231	207	77
Y2	53 YY 69/747	85.3	-2.2	76.7	244	212	0
Y3	42YY 64/745	83.5	3.5	76	248	203	0
Y4	37YY 61/877	81.7	6.8	87.4	249	196	0
Y10	20YY 40/608	69.5	13.8	64.9	219	158	6
Y11	10YY 37/654	67.5	21.1	69.4	224	148	0
O9	28YY 63/746	83.8	12.8	79.4	255	198	0
Y9	40YY 48/750	75.8	5.4	79.9	227	181	0

Yellow/orange 2

SCD code	Dulux code	L*a*b* (lab colour space readings)			RBG		
		L	a	b	R	B	G
O5	06YY 49/797	74.8	26.0	83.6	255	163	0
O10	09YY 57/689	81.0	21.3	71.8	255	184	26
O12	97YR 44/642	72.7	29.3	64.4	252	155	31

Orange 3

SCD code	Dulux code	L*a*b* (lab colour space readings)			RBG		
		L	a	b	R	B	G
O1	68YR 34/780	65	49.5	70.5	255	115	0
O6	91YR 43/816	70.9	38.5	79.7	255	142	0
O7	81YR 38/807	67.5	43	73.7	255	128	0
O8	67YR 28/701	61.2	47.1	62.1	239	107	10

Orange/red 4

SCD code	Dulux code	L*a*b* (lab colour space readings)			RBG		
		L	a	b	R	B	G
O2	55YR 28/778	60.5	56.2	64.7	248	94	1
O3	44YR 26/756	57.8	59.8	60.2	243	81	15
R9	54YR 24/666	56.5	47.5	55.4	224	94	21
R10	50YR 21/603	53.3	49.8	49.9	216	83	29

Red 5

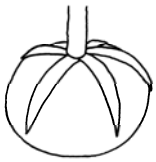
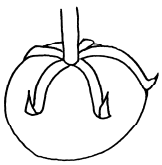
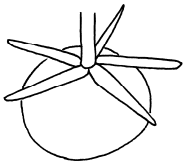
SCD code	Dulux code	L*a*b* (lab colour space readings)			RBG		
		L	a	b	R	B	G
O4	33YR 20/708	51.3	62.4	51.5	225	57	25
R1	26YR 14/548	44.6	48.4	36.2	186	61	38
R2	19YR 13/558	40.6	56.1	35.6	182	35	33
R5	28YR 19/621	50.7	56.4	42.2	215	66	41
R6	31YR 18/648	48.3	60.7	46.9	213	51	28
R8	19YR 14/629	43.9	63.6	40.8	202	25	32
R12	24YR 12/447	38.9	45.1	32.2	164	51	33

Sepal angle

Sepal angle was also a categorical character. This character describes the different angles that the sepals are found in relation to the ripe fruit (see Figure 3.5). Appressed scored as 1, appressed with the sepal tips reflexed 1.5, straight scored as 2 and straight with reflexed sepal tips scored as 2.5 and finally reflexed as 3. If this research were to be repeated, the angle of the sepal (at a designated point along the sepal length) could be measured in degrees in relation to the fruit.

Fruit colour and sepal angle are both categorical/ranked variables but they are ordered in a continuous and meaningful sequence - ordinal. It is important that these characters are included due to their importance for the identification of hybrids in the field. Thus the results can be useful for conservation work on the Galápagos Islands. For these reasons they have been included in the PCO. However when assumptions for statistical analysis are violated results should perhaps be viewed with caution (Dytham, 2003).

Figure 3.5 Sepal score, description and illustration

Sepal score and description	Sepal angle
1) Sepal appressed	
1.5) Sepal appressed/reflexed	
2) Sepal Straight	
2.5) Sepal straight/reflexed	
3) Sepal Reflexed	

Statistical tests

Normality was undertaken in PAST 1.89 (Hammer *et al*., 2009) using two different tests – Shapiro-Wilk and Jarque-Bera in PAST.

ANOVA was undertaken on the normally distributed characters in PAST and TUKEY's (also in PAST) calculated to give a pairwise comparison between, in this case, each of the taxa within each of the morphological characters. The non-normally distributed characters were analysed using a non-parametric test called the Kruskal-Wallis also in PAST, a Mann-Whitney pairwise comparison was also undertaken. All characters were logged in PAST prior to undertaking PCO. The PCO was undertaken in PAST using the Gower similarity measure. The data for PCO needs to be measured, counted or presence/absence data

(Hammer *et al*., 2001 http://folk.uio.no/ohammer/past/past.pdf). PCO analysis was carried out on the hybrid populations in order to determine whether taxa could be distinguished via morphology and whether this correlated with allozyme and STRUCTURE assignment.

Case study of a single SCD accession number 625 and offspring

Leaf samples of tomatoes for the purposes of allozyme electrophoresis need to be collected fresh from living plants as the process is run on non-denatured proteins (Lowe *et al*., 2004). For this reason plants were grown in the Chelsea Physic Garden greenhouses near the NHM laboratories. However it was possible to collect several individual leaf samples from the hybrid area on the Baltra Road on Isla Santa Cruz on the last day of fieldwork. These samples were then taken immediately to the lab and processed and frozen to -80ºC. One individual SCD 625-0 (the 0 denotes that it was a field collected sample) was identified in the field as *S. cheesmaniae*. In addition to this 'parent plant' five offspring plants were grown from seeds collected from this plant in the field and leaf samples were taken from these plants (625-1 to 625-5). The results from the allozyme electrophoresis scoring for this one accession and its offspring were then analysed using STRUCTURE with the other Bulk sample analysis from these hybrid populations.

Stigma Exsertion

The stigma exsertion, or level as it is sometime described, is defined as the difference between the pistil length and the stamen length (Fernandez-Muñoz and Cuartero, 1991). The measurements taken in the field of the stigma exsertion for *S. cheesmaniae*, *S. pimpinellifolium* and S. cheesmaniae x *S. pimpinellifolium* from the Basura population, *S. cheesmaniae* from El Lagoon de Manzanilla (Isla Isabela) and *S. pimpinellifolium* from Garapaterra Road (Isla Santa Cruz) were analysed for comparison. For each of these five groups the stigma exsertion lengths were put into five different groups according to the level of exsertion – 1) not exserted, 2) exsertion - 0.1-0.4mm, 3) exsertion 0.5-0.9mm, 4) exsertion 1.0-1.9mm and 5) exsertion >2mm. The measurements were then represented in pie charts to represent each species at each of the localities.

Timing of flowering

It was observed that several plants had finished flowering at the time of collections from the Baltra Road populations on Santa Cruz. Two trips involved collecting at the Basura (pop 7) on 14.11.02 and 25.10.02. During this time it was noted that some individual plants had finished flowering and just bore fruit. The date at which individual plants had finished flowering was recorded for all taxa belonging to the Basura population along the Baltra Road on Santa Cruz (2002). Most collections at this site took place on one of two dates and the numbers of each of the three taxa that had finished flowering for these two dates was recorded. A percentage calculated and a total number of each species that had finished flowering at the time of collection was calculated and is represented in Table 3.22.

Results

Frequency tables

Tables 3.14 give frequency of alleles at variable loci for each of the different hybrid populations under investigation.

Within-population genetic diversity measures

The within-population genetic diversity measures (Table 3.15-3.18) show that there is very little diversity even in the hybrid populations of tomatoes in the Galápagos Islands. The N_e is highest in *S. cheesmaniae* from the hybrid populations at 0.07 (Table 3.15). This is much higher than in the pure populations found elsewhere in the islands.

Table 3.14.1 Frequency of variable loci in Santa Cruz populations of *S. cheesmaniae*, *S. galapagense* and *S. cheesmaniae* x *S. galapagense*

Taxa		*S. che*	*S. gal*	*S.chexS.gal*	
Year		2002	2002	2002	
		Isabela			
Population	Alleles	El Lagoon de Manzanilla	El Lagoon de Manzanilla	El Lagoon de Manzanilla	Punta Cristóbal
Pop No.		301	301	301	306
Locus					
PGI-2	n	24	23	3	1
	160	.	.	.	.
	145	.	.	.	.
	130	.	0.52	0.33	.
	121	.	.	.	.
	100	1.00	0.48	0.67	1.00
PGM-1	n	24	23	3	1
	106	.	.	.	.
	100	1.00		1.00	1.00
PGM-2	n	24	23	2	1
	129	.	.	.	.
	113	1.00	1.00	1.00	1.00
	100	.	.	.	.
MDH-3	n	24	23	3	0
	104	.	0.96	0.50	.
	100	1.00	0.04	0.50	.
	79	.	.	.	.
IDH-1	n	24	23	3	1
	100	1.00	1.00	1.00	1.00
	95	.	.	.	
6-PGD-2	n	24	23	3	1
	103	.	.	.	
	100	1.00	1.00	1.00	1.00
	82	.	.	.	
DIA-1	n	24	23	3	1
	110	.	.		
	100	1.00	0.33	0.50	1.00
	85	.	0.67	0.50	
DIA-2	n	24	23	3	1
	115	.	.		
	108	1.00	0.96	1.00	1.00
	100		0.04		
DIA-3	n	24	23	3	1
	132	.	.		
	100	1.00	1.00	1.00	1.00

Table 3.14.2 Loci, alleles and allele frequencies in *S. pimpinellifolium* x *S. lycopersicum* from Isla San Cristóbal, *S. pimpinellifolium* from El Chato on Isla Santa Cruz, and *S. lycopersicum* from San Tomás on Isla Isabela.

Taxa		*S. pimp*	*S. lyc*	*S.pimp* x *S. lyc*
Year		2000	2000	2000
		Santa Cruz	Isabela	San Cristóbal.
Population	Alleles	El Chato	San Tomás	Puerto Baquerizo
Pop No.		110	302	
Locus				
PGI-2	**n**	**22**	**5**	**2**
	160	.	.	.
	145	.	.	.
	130	.	.	.
	121	.	.	.
	100	1.00	1.00	1.00
PGM-1	**n**	**25**	**4**	**3**
	106	0.96	.	
	100	0.04	1.00	1.00
PGM-2	**n**	**22**	**5**	**2**
	129	.	.	
	113	.	.	
	100	1.00	1.00	1.00
MDH-3	**n**	**25**	**5**	**3**
	104	.	.	
	100	1.00	1.00	1.00
	79	.	.	
IDH-1	**n**	**22**	**5**	**2**
	100	1.00	1.00	1.00
	95	.	.	
6-PGD-2	**n**	**25**	**5**	**3**
	103	.	.	
	100	1.00	1.00	1.00
	82	.	.	
DIA-1	**n**	**25**	**5**	**3**
	110	.	.	
	100	1.00	1.00	1.00
	85	.	.	
DIA-2	**n**	**25**	**5**	**3**
	115	.	.	.
	108	0.02	1.00	0.33
	100	0.98	.	0.67
DIA-3	**n**	**25**	**5**	**3**
	132	.	.	
	100	1.00	1.00	1.00

Table 3.14.3 Loci, alleles and allele frequencies in Santa Cruz populations of *S. cheesmaniae*, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium*, *S. cheesmaniae* from El Lagoon de Manzanilla (Isabela) and *S. cheesmaniae* from the north coast of Santa Cruz.

Taxa		*S. cheesmaniae*					*S. pimpinellifolium*												*S. che x S. pimp*			
Year		02	00	2002			2000				2002								2002			
		Isabela	Santa Cruz				Santa Cruz				Santa Cruz								Santa Cruz			
Population	Alleles	El Lagoon de Manzanilla	North coast	Zone of Birds	Mina Negra	Basura	Los Gemelos	El Chato	Puerto Ayora	Old Basura	Basura	Mina Roja	Los Gemelos	El Chato	Bella Vista	Garrapatero Rd	Garrapatero Rd	Puerto Ayora	Zone of Birds	Mina Negra	Basura	Mina Roja
Pop No.		301	101-104	105	106	107	109	110	114	115	107	108	109	110	111	112	113	114	105	106	107	108
Locus																						
PGI-2	n	24	30	1	14	19	9	22	5	1	35	5	3	2	1	6	1	1	1	9	36	3
	160	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	145	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	130	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	121	.	.	.	0.07	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0.04	.
	100	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96	1.00
PGM-1	n	24	27	1	14	19	7	25	6	1	35	5	3	2	1	6	1	1	1	9	36	3
	106	.	.	.	0.07	.	0.86	0.96	0.42	1.00	0.03	0.30	.	1.00	.	0.17	1.00	1.00	.	0.06	0.22	.
	100	1.00	1.00	1.00	0.93	1.00	0.14	0.04	0.58	.	0.70	0.70	1.00	.	1.00	0.83	.	.	1.00	0.94	0.78	1.00
PGM-2	n	24	27	1	14	19	8	22	5	1	35	5	3	2	1	6	1	1	1	9	36	3
	129	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	113	1.00	1.00	0.50	0.82	0.68	.	.	.	.	.	.	.	.	.	.	.	.	1.00	0.50	0.39	.
	100	.	.	0.50	0.18	0.32	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.	0.50	0.61	1.00
MDH-3	n	24	30	1	14	19	9	25	6	1	35	5	3	2	1	6	1	1	1	9	36	3
	104	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	79	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
IDH-1	n	24	30	1	14	19	8	22	5	1	35	5	3	2	1	6	1	1	1	9	36	3
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	95	.	0.30	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6-PGD-2	n	24	30	1	14	19	9	25	6	1	35	5	3	2	1	6	1	1	1	9	36	3
	103	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	82	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DIA-1	n	24	30	1	14	19	9	25	6	1	35	5	3	2	1	6	1	1	1	9	36	3
	110	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	85	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
DIA-2	n	24	30	0	14	19	9	25	6	1	26	5	3	1	1	4	1	1	0	4	29	3
	115	.	.		.	0.06	.	.	.	.	.	.	.	.	.	.	.	.		.	.	.
	108	1.00	0.98		0.82	0.50	.	0.02	.	.	0.02	0.30	.	.	.	.	.	.	.	.	0.21	0.17
	100		0.02		0.18	0.44	1.00	0.98	1.00	1.00	0.98	0.70	1.00	1.00	1.00	1.00	1.00	1.00	.	1.00	0.79	0.83
DIA-3	n	24	30	1	14	19	9	25	6	1	35	5	3	2	1	6	1	1	1	9	36	3
	132	.	.	.	0.07	.	.	.	.	.	0.03	.	.	.	.	.	.	.	.	.	.	.
	100	1.00	1.00	1.00	0.93	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Legend to Table 3.14

	Private to *S. cheesmaniae*
	Private to *S. cheesmaniae and S. galapagense*
	Private to *S. pimpinellifolium*
	Private to *S. cheesmaniae, S. galapagense and S. lycopersicum*
	Indications of hybridization -*S. cheesmaniae x S. pimpinellifolium*
	Indications of hybridization -*S. cheesmaniae x S. galapagense*
	Indications of hybridization with *S. pimpinellifolium*

Table 3.15. Summary of within population genetic diversity measures for all taxa and their hybrids within the
Galápagos Islands (SCD individual).

N_e (effective no alleles)

N_a (obs. no. alleles Kimura and Crow 1964)

I (Shannon's information index (Lewontin 1972)

H_O (Observed heterozygosity Levene 1949)

H_e (Expected heterozygosity Levene 1949)

Nei (1976) expected heterozygosity

Pop name	No. isles	No. pop.	n.	Year	N_a	N_e	I	H_o	H_e	Nei H_e
S. cheesmaniae	6	14	106	2000/02	1.0179	1.0061	0.006	0.0019	0.0046	0.0038
S. galapagense	5	9	98	2000/02	1.0357	1.0171	0.0164	0.004	0.0116	0.0106
S. pimp-nellifolium	1	14	55	2000/02	1.028	1.0102	0.0101	0.001	0.0068	0.0063
S. lycopersicum	1	3	10	2000/02	1	1	0	0	0	0
S. pimp-inellifolium Basura (2000)	1	1	10	2000	1.05	1.0441	0.0331	0.0125	0.025	0.0234
S. cheesmaniae (hybrid pops)	1	3	34	2002	1.151	1.072	0.0655	0.028167	0.0514	0.0415
S. pimp-inellifolium (hybrid pops)	1	2	40	2002	1.15	1.0567	0.05135	0.0145	0.0364	0.0338
S. cheesmaniae x S. pimp-inellifolium (hybrid pops)	1	4	49	2002	1.088	1.0439	0.0405	0.0179	0.0278	0.0264
S. cheesmaniae x S. galapagense	1	2	4	2002	1.075	1.07	0.0506	0.0167	0.0434	0.0361
S. pimp-inellifolium x S. lycopersicum	1	1	7	2000	1.1	1.0239	0.0334	0.0071	0.0203	0.0189

Table 3.16. Within population genetic diversity measures for *S. cheesmaniae, S. pimpinellifolium* and *S. cheesmaniae x S. pimpinellifolium* hybrid populations from Santa Cruz (SCD individual)

Pop name	Pop no.	No. pop.	n	year	N_a	N_e	I	H_o	H_e	Nei
Santa Cruz										
Zone of Birds										
S. cheesmaniae	105		1	2002	1.1	1.0526	0.0365	0.0526	0.0526	0.0263
S. cheesmaniae x S. pimpinellifolium	105		1	2002	1	1	0	0	0	0
Mean	105	**2**	**2**		**1**	**1.0263**	**0.01825**	**0.0263**	**0.0263**	**0.01315**
Mina Negra										
S. cheesmaniae.	106		14	2002	1.3	1.0645	0.0855	0.0214	0.0511	0.0492
S. cheesmaniae x S. pimpinellifolium	106		9	2002	1.1	1.0559	0.0454	0.0222	0.032	0.0302
Mean		**2**	**23**		**1.2**	**1.0602**	**0.06545**	**0.0218**	**0.04155**	**0.0397**
Basura										
S. pimpinellifolium	107		10	2000	1.1	1.0441	0.0331	0.0125	0.025	0.0234
S. cheesmaniae	107		19	2002	1.2	1.099	0.0746	0.0105	0.0504	0.0491
S. pimpinellifolium	107		35	2002	1.2	1.041	0.0416	0.009	0.026	0.0256
S. cheesmaniae x S. pimpinellifolium	107		36	2002	1.2	1.1005	0.094	0.0326	0.0624	0.0615
Mean		**4**	**100**		**1.1**	**1.07115**	**0.06083**	**0.01615**	**0.04095**	**0.0399**
Mina Roja										
S. pimpinellifolium	108		7	2000	1.1	1.03	0.0281	0.0083	0.0205	0.0187
S. pimpinellifolium	108		5	2002	1.1	1.0724	0.0611	0.02	0.0467	0.042
S. cheesmaniae x S. pimpinellifolium	108		3	2002	1.1	1.0192	0.0225	0.0167	0.0167	0.0139
Mean		**3**	**15**		**1.1**	**1.04053**	**0.03723**	**0.015**	**0.02797**	**0.02487**
Mean for overall hybrid zone		**11**	**140**		**1.1**	**1.05265**	**0.04749**	**0.01871**	**0.03485**	**0.0309**

The results show that there is genetic support for the evidence found for hybrids between the *S. cheesmaniae* and *S. galapagense* on Isla Isabela (Table 3.14). Furthermore, there is genetic support for the evidence found for hybrids between the *S. pimpinellifolium* and *S. lycopersicum* on Isla San Cristóbal (Table 3.14). Table 3.16 shows the summary of the within population diversity for the Isla Santa Cruz hybrid populations between *S. cheesmaniae* x *S. pimpinellifolium* along the Baltra Road.

Table 3.17. Within population genetic diversity measures for *S. cheesmaniae x S. galapagense* on Isla Isabela (SCD individual)

Pop name	Pop no.	No. pop.	n	year	N_a	N_e	I	H_O	H_E	Nei
Villamil	301	1	3	2002	1.2	1.14	0.1011	0.033	0.087	0.0722
Punta Cristóbal	306	1	1	2002	1	1	0	0	0	0
Mean *S. cheesmaniae x S. galapagense*	**3**	**2**	**4**		**1.1**	**1.07**	**0.05055**	**0.01665**	**0.04335**	**0.0361**

Table 3.18. Within population genetic diversity measures for *S. pimpinellifolium* x *S. lycopersicum* on Isla San Cristóbal (SCD individual)

Pop name	Pop no.	No. pop.	n	year	N_a	N_e	I	H_O	H_e	Nei
Puerto Baquerizo	901	1	7	2000	1.1	1.0239	0.0334	0.0071	0.0203	0.0189
Mean *S. pimpinellifolium* x *S. lycopersicum*	9	1	7	2000	1.1	1.0239	0.0334	0.0071	0.0203	0.0189

The results show that the hybrid populations are more diverse (without exception) than the pure taxa. The peak of diversity is found in the Basura population where N_e reached 1.1 in *S. cheesmaniae* x *S. pimpinellifolium.* The Basura population is the most diverse of all of the tomato populations in the Galápagos (see Table 3.16 for details).

A mean of the different hybrid groups show that the hybrids have a higher N_e than any of the "pure taxa". The mean for *Solanum cheesmaniae* x S. *galapagense* has the highest N_e at 1.07 (Table 3.17) and *S. pimpinellifolium* x *S. lycopersicum* the lowest N_e of 1.0239 (Table 3.18).

Morphological character statistics

Normality was tested using Shapiro-Wilk and Jarque-Bera in PAST (Hammer *et al.* 2001).

Table 3.19. Morphological characters and results from normal distribution test. Table indicates those that do *not* deviate from normal. K-W = Kruskal-Wallis Pairwise Comparison as a non-parametric alternative to ANOVA.

Legend

1 = *S. cheesmaniae* from El Lagoon

2 = *S. cheesmaniae* from the hybrid populations

3 = *S. pimpinellifolium* from the hybrid populations

4 = *S. cheemaniae* x *S. pimpinellifolium* hybrids

5 = *S. pimpinellifolium* from Garapaterra Road

code	Morphological character	Data type	Shapiro-Wilk (P>0.05)	Jarque-Bera (P>0.05)	Test
1	Flower A : Length from centre of the corolla to apex of the corolla lobe (mm)	Continuous	3,4,5	2,3,4,5	ANOVA
2	Flower B: Length of the corolla lobe from apex to the corolla lobe junction (mm)	Continuous	2,5	all	ANOVA
3	Flower C: Length from the centre of the corolla to the corolla lobe junction (mm)	Continuous	1,5	all	ANOVA
4	Flower D: Corolla lobe width (mm)	Continuous	1,2,5	1,2,4,5	ANOVA
5	Anther length	Continuous	2	1,2,4,5	ANOVA
6	Stigma exsertion length (1 is all the same)	Continuous discrete	2,3	2,3,4,5	K-W
7	Sepal length on flower	Continuous	1,2	all	ANOVA
8	Sepal width on flower (1 is all the same)	Continuous	none	none	K-W
9	Inflorescence length	Continuous	4,5,	1,3,4,5	K-W
10	Flower number per inflorescence	Continuous discrete		2, 4, 5	K-W
11	Fruit size	Continuous	all	all	ANOVA
12	Fruit skin thickness	Continuous	5	all	ANOVA
13	Fruit colour	Ordinal	/	/	/
14	Seed number per fruit	Continuous discrete	1,2,3,4,	all	ANOVA
15	Sepal length on fruit	Continuous	1,2,	all	K-W
16	Sepal width on fruit	Continuous	none	1,3,4,5	K-W
17	Sepal angle on fruit (5 is all the same)	Ordinal	none	2	K-W

I then tested for differences between taxa and hybrids for morphological characters.

Tables 3.20. Results of the analysis of morphological characters using ANOVA and Tukey's pairwise comparison on the normally distributed characters and Kruskal-Wallis and Mann-Whitney Pairwise Comparison as a non-parametric alternative. (All undertaken in PAST).

Highlighted cells indicate the taxa that were found to be significantly different from other corresponding taxa. Red indicates that the test was undertaken by Tukey's pairwise comparison and orange indicates that the test was undertaken with non-parametric Mann- Whitney pairwise comparisons due to deviation from normality.

Legend

pure che = *S. cheesmaniae* from El Lagoon de Manzanilla (Isla Isabela)

che (hyb) = *S. cheesmaniae* from the hybrid populations along the Baltra Road (Isla Santa Cruz)

pimp (hyb) = *S. pimpinellifolium* from the hybrid populations along the Baltra Road (Isla Santa Cruz)

che x pimp = *S. cheesmaniae* x *S. pimpinellifolium* from the hybrid populations along the Baltra Road (Isla Santa Cruz)

pure pimp = *S. pimpinellifolium* from the Garapaterra Road, (Isla Santa Cruz)

Table 3.20.1. Flower A: Length from centre of the corolla to apex of the corolla lobe (mm)

Levene p=<0.01, Welch ANOVA p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che					<0.05
che (hyb)					<0.05
pimp (hyb)					<0.05
che x pimp					<0.05
pure pimp					

Table 3.20.2. Flower B: Length of the corolla lobe from apex to the corolla lobe junction (mm)

Levene p=<0.01, Welch ANOVA p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che					<0.05
che (hyb)					<0.05
pimp (hyb)					<0.05
che x pimp					<0.05
pure pimp					

Table 3.20.3. Flower C: Length from the centre of the corolla to the corolla lobe junction (mm). Welch ANOVA p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che			<0.05		
che (hyb)			<0.05		
pimp (hyb)					<0.05
che x pimp					
pure pimp					

Table 3.20.4. Flower D Corolla lobe width (mm). Welch ANOVA p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che			<0.05		
che (hyb)					<0.05
pimp (hyb)				<0.05	<0.05
che x pimp					
pure pimp					

Table 3.20.5. Anther Length (mm). Welch ANOVA p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che		<0.05		<0.05	<0.05
che (hyb)					
pimp (hyb)					<0.05
che x pimp					
pure pimp					

Table 3.20.6. Stigma exsertion (mm). Kruskal-Wallis p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che		<0.05	<0.05	<0.05	<0.05
che (hyb)					
pimp (hyb)					<0.05
che x pimp					<0.05
pure pimp					

Table 3.20.7. Sepal Length on Flower (mm). Welch ANOVA p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che		<0.05	<0.05		
che (hyb)					
pimp (hyb)					
che x pimp					
pure pimp					

Table 3.20.8. Sepal width on flower (mm). Not significant with Kruskal-Wallis

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che					
che (hyb)					
pimp (hyb)					
che x pimp					
pure pimp					

Table 3.20.9. Inflorescence Length (mm). Kruskal-Wallis p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che			<0.05		
che (hyb)			<0.05	<0.05	
pimp (hyb)					<0.05
che x pimp					<0.05
pure pimp					

Table 3.20.10. Flower number per inflorescence. Kruskal-Wallis p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che			<0.05	<0.05	
che (hyb)			<0.05	<0.05	
pimp (hyb)					<0.05
che x pimp					
pure pimp					

Table 3.20.11 Fruit Size (mm). Welch ANOVA p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che			<0.05		<0.05
che (hyb)			<0.05	<0.05	<0.05
pimp (hyb)					<0.05
che x pimp					<0.05
pure pimp					

Table 3.20.12. Skin thickness of fruit (mm). Welch ANOVA p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che					<0.05
che (hyb)					<0.05
pimp (hyb)					<0.05
che x pimp					<0.05
pure pimp					

Table 3.20.13. Fruit Colour. Kruskal-Wallis p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che			<0.05	<0.05	<0.05
che (hyb)			<0.05	<0.05	<0.05
pimp (hyb)				<0.05	
che x pimp					<0.05
pure pimp					

Table 3.20.14. Seed number per fruit. Welch ANOVA p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che		<0.05	<0.05		<0.05
che (hyb)			<0.05	<0.05	<0.05
pimp (hyb)					
che x pimp					<0.05
pure pimp					

Table 3.20.15. Sepal length on Fruit (mm). Not significant with Kriskal-Wallis

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che			<0.05		
che (hyb)					
pimp (hyb)					
che x pimp					
pure pimp					

Table 3.20.16. Sepal width on Fruit (mm). Welch ANOVA p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che		<0.05	<0.05	<0.05	<0.05
che (hyb)					
pimp (hyb)					<0.05
che x pimp					
pure pimp					

Table 3.20.17. Sepal Angle. Kruskal-Wallis p=<0.01

	pure che	che (hyb)	pimp (hyb)	che x pimp	pure pimp
pure che		<0.05	<0.05	<0.05	<0.05
che (hyb)			<0.05	<0.05	<0.05
pimp (hyb)					
che x pimp					
pure pimp					

The best morphological characters to discriminate the different taxa using ANOVA and TUKEY's pairwise comparison and Mann-Whitney were found to be the fruit characters – fruit size, fruit colour, sepal angle on fruit and seed number per fruit.

Comparison between parental phenotype and offspring genotype

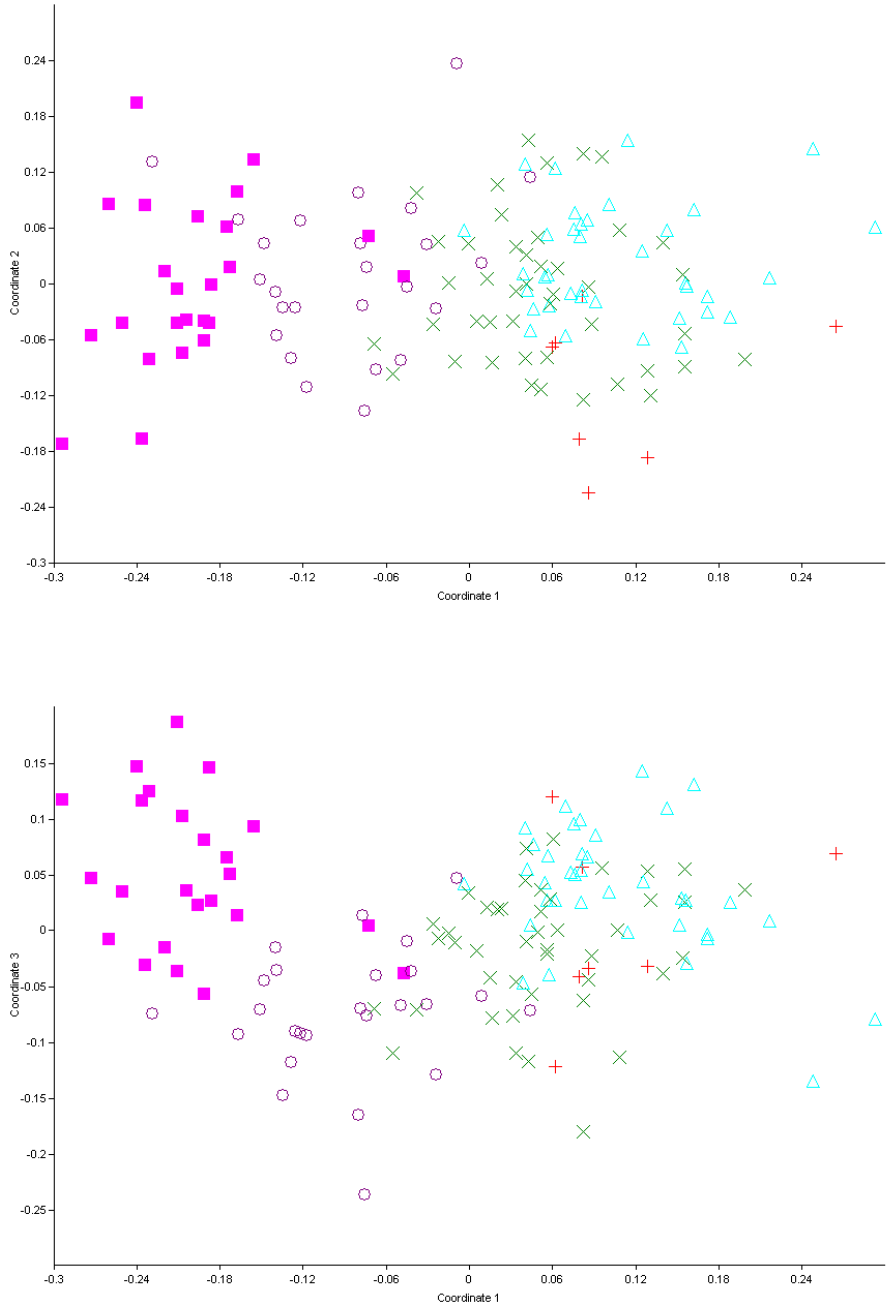
Tests were carried out to establish if the parental phenotype and the offspring genotype assignments were significantly different by comparing the field morphological species circumscription and the assignment generated using the STRUCTURE results for two different thresholds (cut off points) (*Tq*). Having tested for normal distribution and found that the data were not normally distributed I undertook a non-parametric Wilcoxon test (PAST). The results from this test showed that the only pairs that showed a significant difference were the field morphological circumscription vs the stricter 93% *Tq* STRUCTURE assignment done on offspring.

Field	vs	85% Tq	P = 0.45	not significant
85% *Tq*	vs	93% Tq	P = 0.08	not significant
Field	vs	93% Tq	P < 0.004	significant

PCO using field circumscription to discriminate groups

Figures 3.6 show PCO of the morphological characters – the symbols represent the field circumscription of the different taxa. The results show some overlap between the taxa but that the hybrids are found to be morphologically intermediate between the *S. cheesmaniae* to the left and the *S. pimpinellifolium* to the right.

Fig. 3.6. PCO (Gower) of morphological characters (field circumscription symbols below)



Legend – symbols as defined from the field circumscription

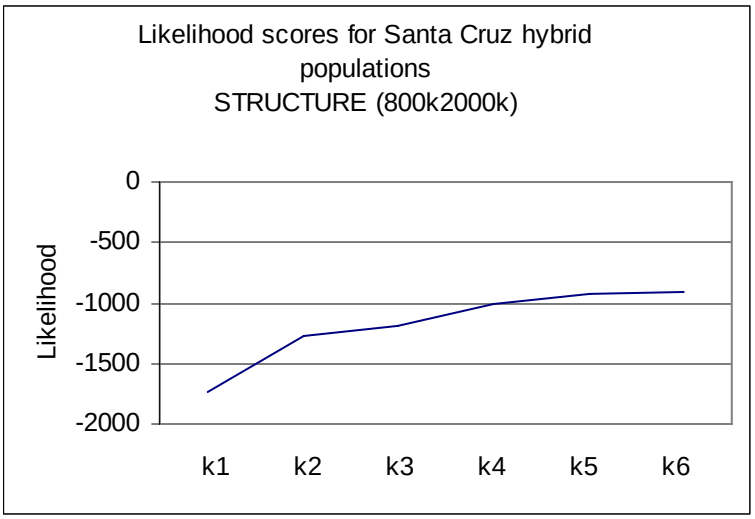
Pink square	*S. cheesmaniae* from El Lagoon de Manzanilla
Purple circle	*S. cheesmaniae* from hybrid populations
Blue triangle	*S. pimpinellifolium* from hybrid populations
Green x	*S. cheesmaniae* x *S. pimpinellifolium*
Red Cross	*S. pimpinellifolium* from Garapaterra Road

STRUCTURE

STRUCTURE (Prichard & Wen, 2003) is used to detect genetic structure within and between populations. It generates clusters (K) that are based on Hardy Weinberg (H-W) disequilibrium and linkage disequilibrium (Kaeuffer *et al*., 2007). I concur with (Burgarella *et al*., 2009) that the best way of describing STRUCTURE is a Bayesian clustering method that does not rely on an *a priori* morphological classification. A random walk (Markov chain Monte Carlo) simulation gives the estimates of the membership of each individual into clusters using posterior probability (Burgarella *et al*., 2009). A series of cluster (K) numbers are then run (e.g. K=1 to K=10) until the most suitable is found. The likelihood score generated in the results acts as a guide to determine the most likely cluster number.

STRUCTURE software gives an assignment probability for each individual from each k category. This was then compared to the morphological classification also undertaken here. The most likely number of clusters (Pritchard *et al*., 2003; Kaeuffer *et al*., 2007) is calculated by analysing the likelihood scores and the graphic produced by the programme. The analysis is repeated eight times to ensure consistency and to check for discrepancies. If all the results are consistent then errors are unlikely to have occurred. A mean of each likelihood score is plotted in Figure 3.7. The most applicable k is reached when the likelihood scores plateau.

Fig. 3.7. Likelihood scores for STRUCTURE (800k2000k) from K1 to K6 (eight runs per K)



Figs 3.8. k2-k4 assignments from STRUCTURE. Santa Cruz hybrid populations with pure *S. cheesmaniae* from El lagoon de Manzanilla (Isla Isabela) and pure *S. pimpinellifolium* from Garapaterra Road (Isla Santa Cruz). This analysis was generated using the bulk data set. Isla Santa Cruz populations: 10502 Zone of Birds; 10602 Mina Negra; 10702 Basura; 10802 Mina Roja; 10902 Los Gemelos; 11202 Garapaterra Road; 11302 Garapaterra Road; Isla Isabela population: 30102 El lagoon de Manzanilla.

Fig. 3.8.1. K2 (800k2000k)

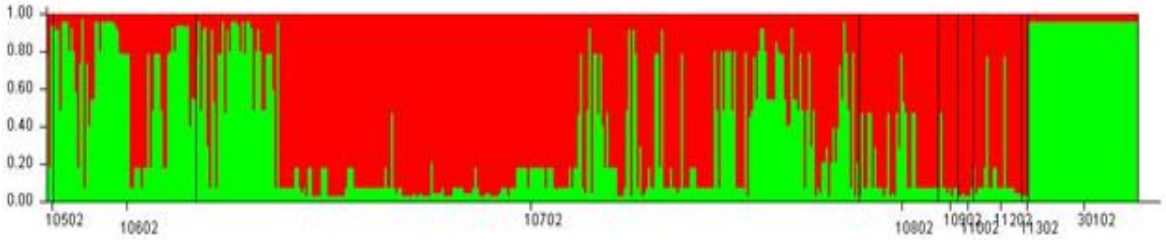


Fig. 3.8.2. K3 (800k2000k)

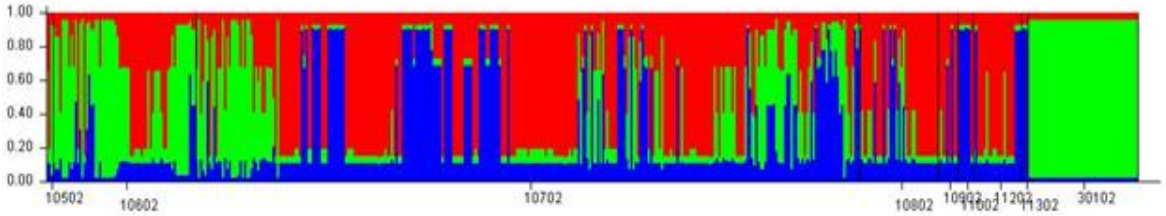


Fig. 3.8.3. K4 (800k2000k)

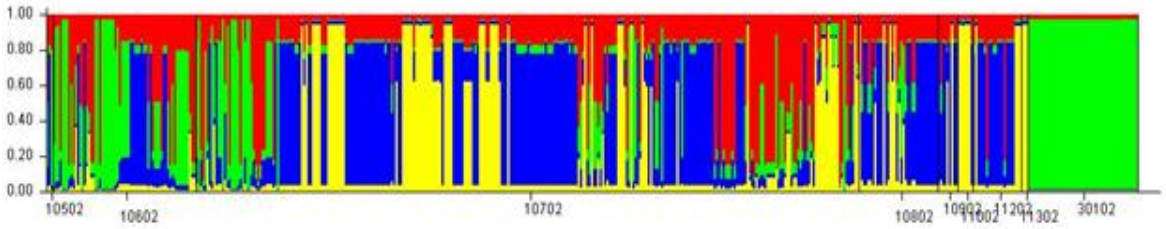
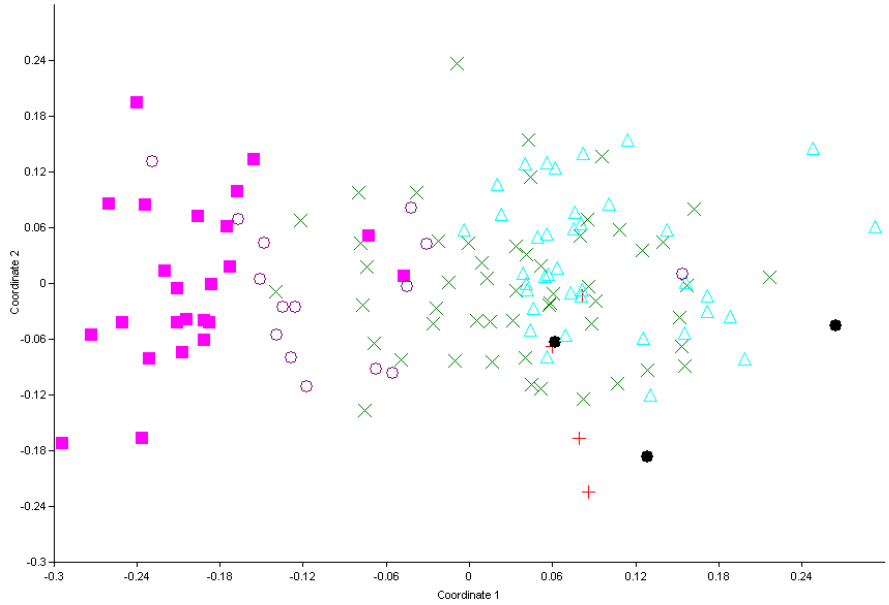
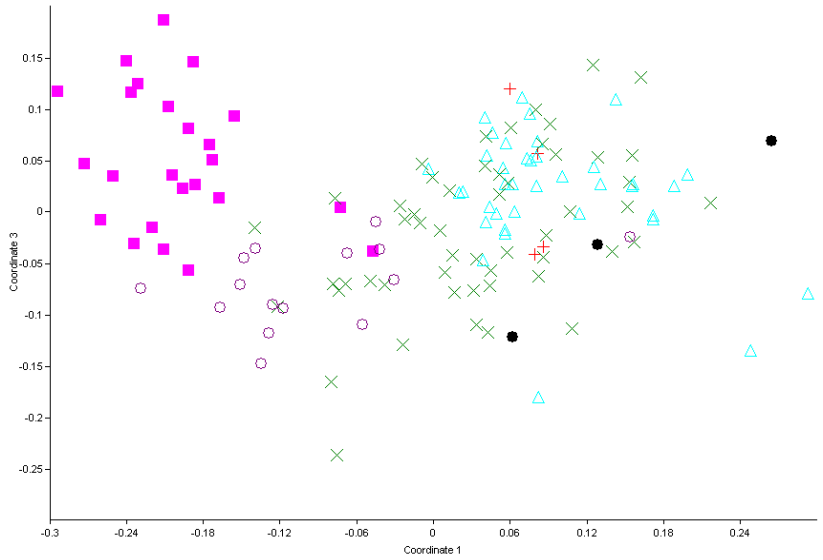


Fig. 3.9. PCO (Gower) of morphological characters STRUCTURE.





Legend – symbols as defined from STRUCTURE assignment

Pink square	*S. cheesmaniae* from El Lagoon de Manzanilla
Purple circle	*S. cheesmaniae* from hybrid populations
Blue triangle	*S. pimpinellifolium* from hybrid populations
Green x	*S. cheesmaniae* x *S. pimpinellifolium*
Red Cross	*S. pimpinellifolium* from Garapaterra Road
Black dot	*S. pimpinellifolium* from Garapaterra Road (putative hybrids with *S. lycopersicum*)

Hybrid 1 STRUCTURE analysis (undertaken on the bulk data set)

The likelihood scores (Fig. 3.7) and the STRUCTURE box plots ((Fig. 3.8) below indicate that the k2 is the most likely. Between k2 and k3 the likelihood scores plateaux, indicating that k2 is the most likely. Likewise the STRUCTURE box plots for K2 are the most meaningful. K3 however is quite interesting as it shows a third taxon appearing in the middle of a group of plants that morphologically were pure *S. pimpinellifolium*, see 10702 (Basura). Given the overall low diversity this might be due to the PGM-1 106 allelic variation (see Table 3.14).

The STRUCTURE assignments generated with the allozyme electrophoresis results were used as a tool to discriminate the taxa in the PCO in Fig. 3.9. The overall look of the PCO is very similar to the PCO that is undertaken with the field circumscription because in general the field circumscription was the same as the STRUCTURE assignment and was found to be within this *Tq* range.

STRUCTURE Analysis undertaken on the individual data set

Hybrid 2 STRUCTURE results for the Santa Cruz hybrids with north coast populations see likelihood graph in Figure 3.10. The STRUCTURE assignment was used to generate pie charts (Figure 3.11) that showed the percentage of each assignment found at each population in the northern part of Santa Cruz. This figure showed that the pure *S. cheesmaniae* are found on the coast and the pure *S. pimpinellifolium* are found in the highlands at 600m. The hybrids are found in the zone between with all three species found at the Basura population.

Fig. 3.10. Likelihood score for the STRUCTURE hybrid work

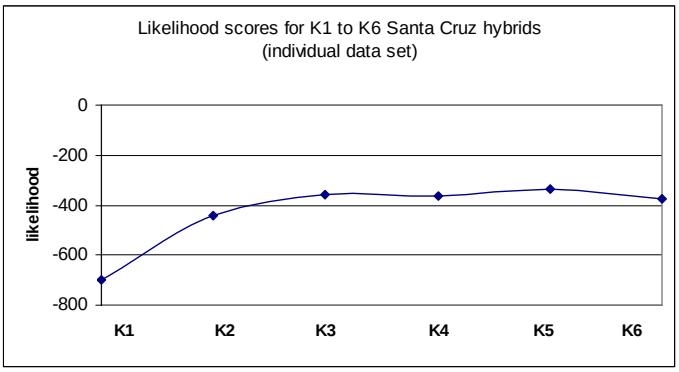


Figure 3.11 shows the pie charts showing the percentage of each taxon in the different areas along the Baltra Road and the north Coast populations

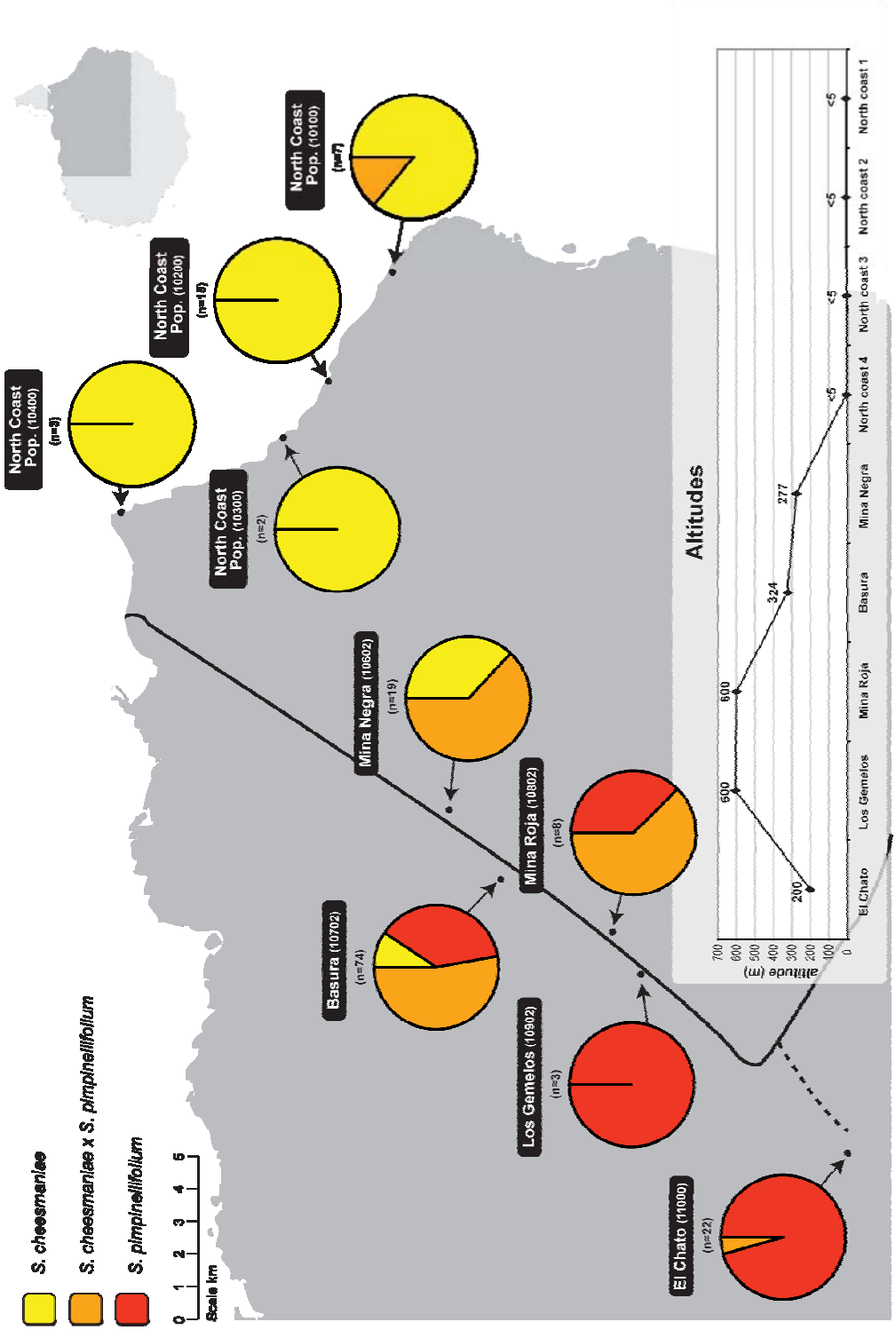


Figure 3.11. Distribution of *Solanum cheesmaniae*, *S. pimpinellifolium* and their hybrids along the Baltra road, Santa Cruz.

Study of one accession and offspring

Table 3.21 shows the genetic diversity of one individual accession (625) and five offspring collected from a single fruit. It clearly shows evidence of high variability, suggesting hybridization and supporting all previous results.

Table 3.21 Detail of the results of the STRUCTURE 2.1 analysis (k2). 'Pure' *S. cheesmaniae* is 0 – 0.15; *S. cheesmaniae x S. pimpinellifolium* is >0.15 to <0.85; 'pure' *S. pimpinellifolium* is 0.85 - 1

	Parent plant - field collected	**Offspring 1 (hybrid)**	**Offspring 2 *S. pimp-inellifolium***	**Offspring 3 *S. chees-maniae***	**Offspring 4 (hybrid)**	**Offspring 5 *S. pimp-inellifolium***
Accession no.	625-0	625-1	625-2	625-3	625-4	625-5
STRUC-TURE Assignment score	0.514	0.708	0.927	0.081	0.465	0.927

Timing of flowering of the different taxa at the Basura Population

A comparison of the timing of the flowering cessation of plants at the Basura population showed that at the time of collection 35% of *S. pimpinellifolium*, 21% of *S. cheesmaniae* x *S. pimpinellifolium*, and 9% of *S. cheesmaniae* had finished flowering (Table 3.22). This can be compared to percentage of mixed offspring of plants that were collected at the Basura Population (107) (Table 3.22).

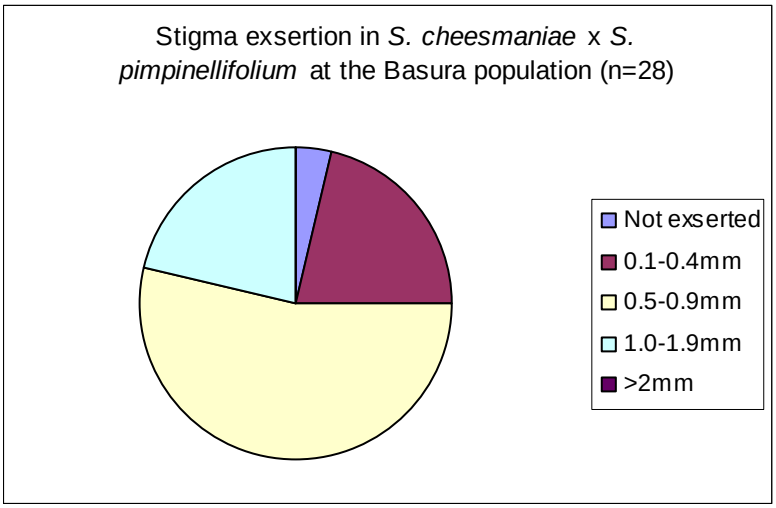
Table 3.22. Differences in flowering time in *S. cheesmaniae*, *S. cheesmaniae* x *S. pimpinellifolium* and *S. pimpinellifolium* at the Basura population along the Baltra Road on Santa Cruz Island.

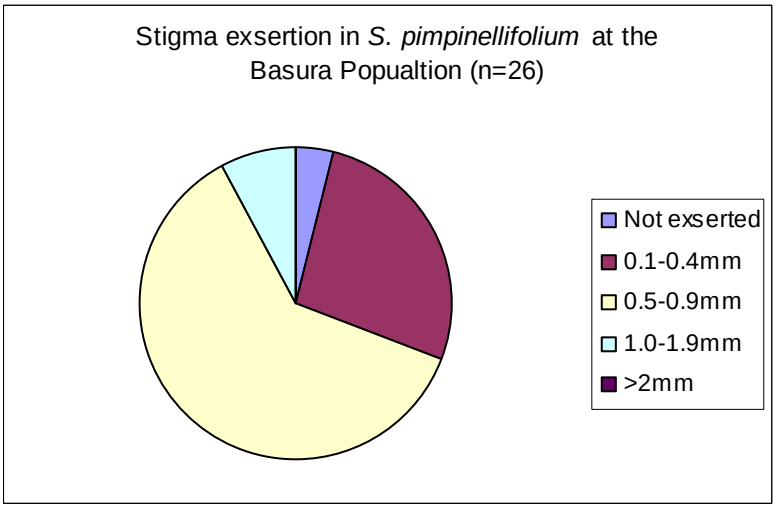
Collection date▼	Taxa ►	*S. cheesmaniae*	*S. cheesmaniae* x *S. pimpinellifolium*	*S. pimpinellifolium*
25.10.02	Total collected	11	11	25
	Flowering finished	1	1	8
	%	9%	9%	32%
14.11.02	Total collected	0	22	8
	Flowering finished	/	7	3
	%	/	32%	38%
Mean 25.10.02 & 14.11.02	Mean %	9%	21%	35%

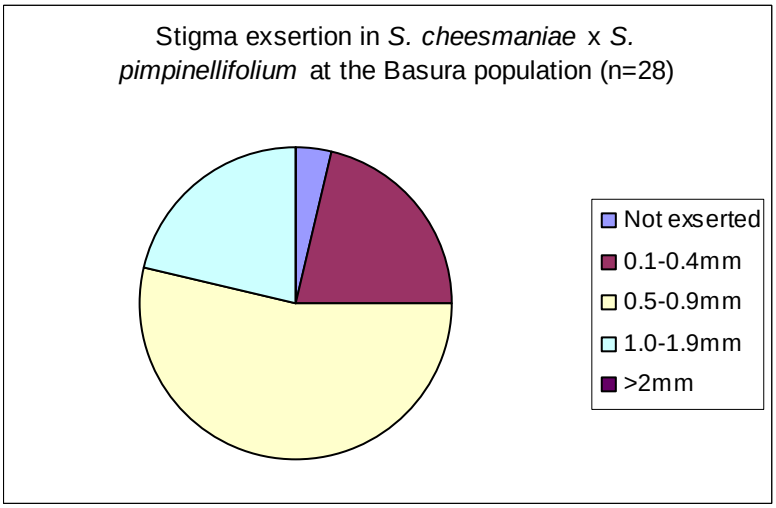
Stigma exsertion

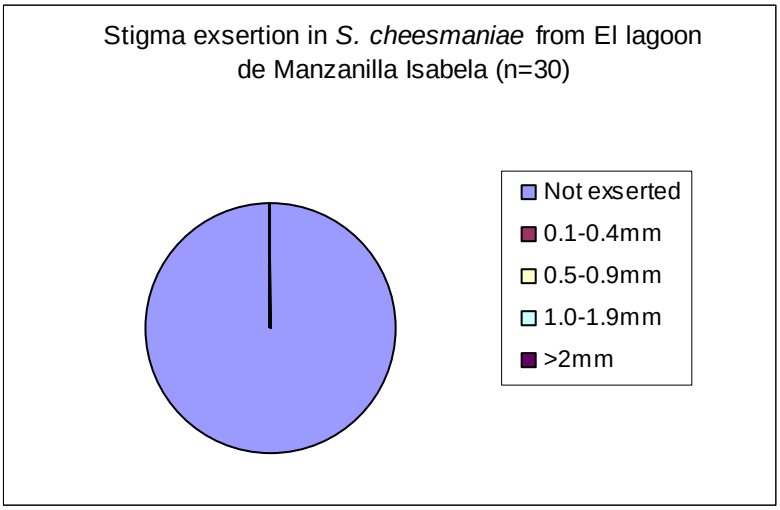
An analysis of the distribution between taxa of flowers with exserted stigmas was undertaken at the Basura population (Isla Santa Cruz). One population was selected to minimize variables. It has been shown that stigma exsertion is not only under genetic control but also can be effected by ambient temperature (for example Fernandez-Muñoz and Cuartero, 1991). Most individuals of *S.cheesmaniae*, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* had flowers with exserted stigmas at the Basura site. *Solanum cheesmaniae* was the only species that had an individual (n=1) with an included stigma. Very few individuals in any taxa in the Basura population had stigmas with over 1.5mm exsertion. These can be compared to the *S. pimpinellifolium* from Garapaterra Road and *S. cheesmaniae* from El Lagoon de Manzanilla (Isla Isabella); where in *S. pimpinellifolium* all individuals had flowers with exserted stigmas and in *S. cheesmaniae* all individuals had flowers with included stigmas (Fig. 3.12).

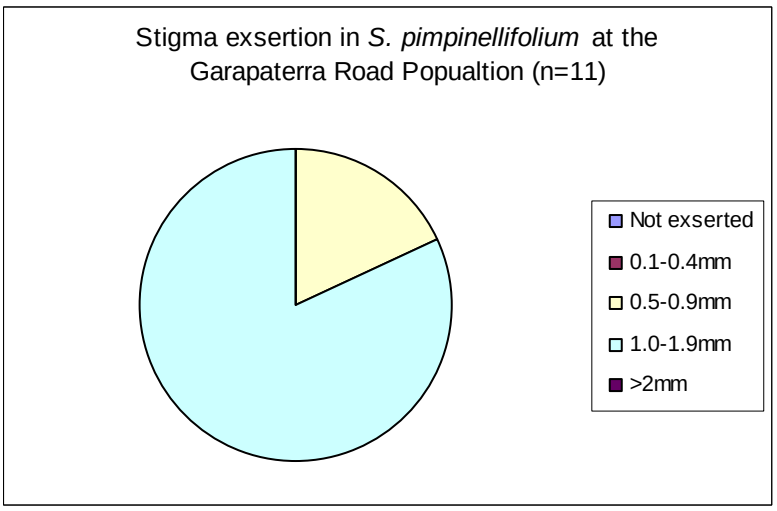
Fig. 3.12. Stigma exsertion for *S. cheesmaniae*, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* from Basura population and *S. cheesmaniae* from El Lagoon de Manzanilla and *S. pimpinellifolium* from the Garapaterra Road populations







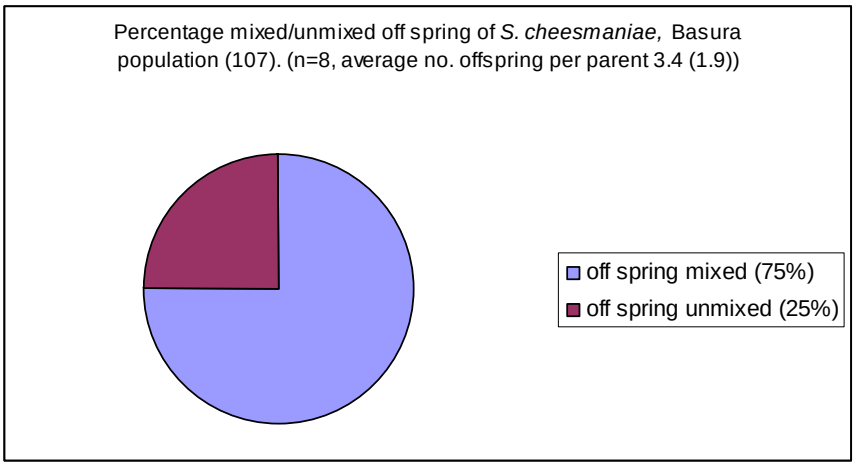


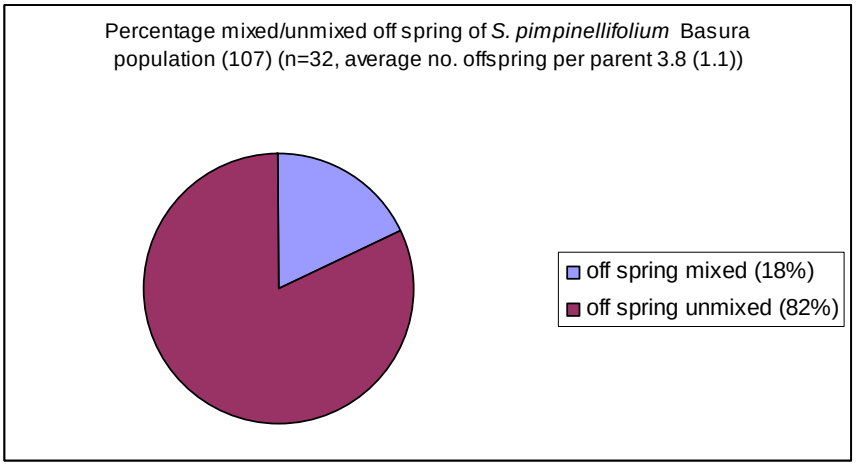


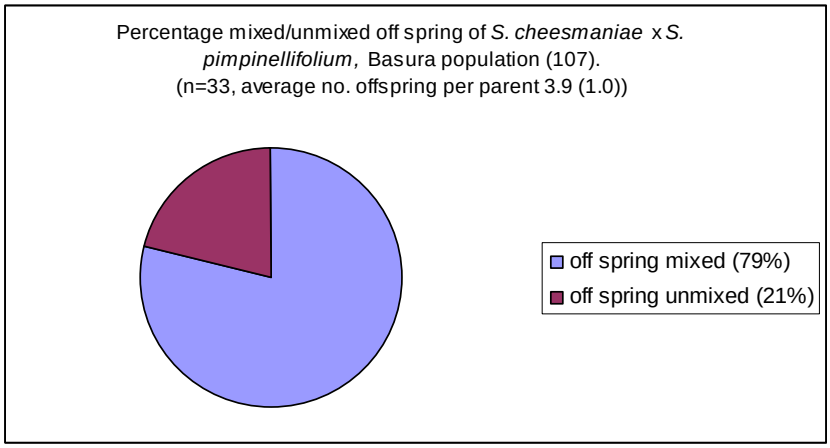
Percentage of mixed offspring per species

Percentage of mixed offspring per species at the Basura was calculated and the results are summarized in Figures 3.13. The results show that the plants that were described as *S. cheesmaniae* x *S. pimpinellifolium* hybrids at the time of collection in the field had more off-spring that was assigned as mixed using allozyme electrophoresis. Plants originally assigned as S. cheesmaniae closely followed this and surprisingly few offspring of *S. pimpinellifolium* individuals were found to be hybrids.

Fig. 3.13 Percentage mixed offspring from field collected accessions that were described in the field as the following Figure A -*S. cheesmaniae*, Fig B *S. pimpinellifolium* and Fig C - *S. cheesmaniae* x *S. pimpinellifolium* hybrids. This is taken from the Basura population.







Discussion

The overall findings are:

1) Allozyme analysis was found to support the morphological evidence of hybrids between several taxa, native and introduced, within the Galápagos Islands.

2) Evidence from allozyme electrophoresis supported the hypothesis of two locations for hybrids on Isla Isabela between the native and endemic *S. cheesmaniae* and *S. galapagense*.

3) Allozymes and morphological data are congruent in weekly supporting the hypothesis of hybridization between the two introduced species – *S. pimpinellifolium* and *S. lycopersicum* from San Cristóbal. Both, allozymes and morphological data provided discontinuous characters that delimit the different species.

4) Finally and most significantly genetic support was found for hybrid populations between native *S. cheesmaniae* and introduced *S. pimpinellifolium* along the Baltra Road. These hybrids were initially identified in the field by having intermediate morphological characters. My data demonstrate that the hybrids have intermediate markers between the two parents and the PCO show that the hybrids 'cluster' in the centre between the two parental taxa along the Baltra Road which in turn cluster between the two 'pure' taxa populations which were included for comparison. Here, individuals designated morphologically as either *S. cheesmaniae* or *S. pimpinellifolium* were found to have offspring that showed evidence of not being purebred. This might indicate the following: 1) that each generation is becoming more introgressed, 2) that these hybrids are not exhibiting morphological intermediate characters (see Allendorf *et al*., 2001) or 3) that morphological characters are no longer the most reliable character when determining the status of tomato populations on the islands. Thus, I found allozymes to be a suitable and better diagnostic tool to detect geneflow, hybridization and introgression.

Hybridization between the endemic taxa

There are only two islands where endemic Galápagos tomatoes occur in sympatry – Isabela and Fernandina. On both these islands evidence of hybridization was found. In this section only individual plants that were identified as intermediate between *S. cheesmaniae* and *S. galapagense* are discussed.

On Isabela, hybrids were found at El Lagoon de Manzanilla and Punta Cristóbal, supporting the results of Rick (1963) and Darwin *et al*. (2003). In these populations the accessions were identified in the field as being morphologically intermediate and this was later supported with the allozymes (see Table 3.14)

In another population at Los Túneles (pop 602, Isla Fernandina) genetic evidence for hybridization was found in only a couple of accessions. In two accession of *S. cheesmaniae*, alleles private to *S. galapagense* were found and conversely in one individual of *S. galapagense* alleles private to *S. cheesmaniae* were found.

All these populations are in areas where *S. cheesmaniae* and *S. galapagense* are found in sympatry. It is also interesting to note that Los Túneles on Isla Fernandina and Punta Cristóbal on Isla Isabela were the only populations of *S. cheesmaniae* (except for the Baltra Road populations) found in the field with over 25% of the flowers measured with exserted stigmas during the 2002 field season.

Rick (1963) found hybrids between the two endemic taxa, which he regarded (following the biological species concept) as the same species. The TGRC database also confirmed accessions of *S. cheesmaniae* and *S. galapagense* in sympatry. Hybridization between closely related taxa on islands appears to be not uncommon. For example in the Galápagos Islands members belonging to the endemic genus *Scalesia* are known to hybridise. For example *Scalesia divisa* and *Scalesia incisa,* native to Isla San Cristóbal, form a hybrid swarm between their ranges. This has been shown using morphological and genetic analysis (Nielsen *et al*., 2003).

Hybridization between introduced taxa: *Solanum pimpinellifolium* and S. *lycopersicum*

There was some evidence of hybridization between *S. pimpinellifolium* and *S. lycopersicum* on San Cristóbal. Here the individual plants looked morphologically intermediate and this was supported by allozymes (Table 3.14). On Santa Cruz where *S. pimpinellifolium* was growing as a ruderal weed along the roadside between Bella Vista and Garapaterra Beach, I found several plants that looked intermediate between these two, particularly in one area where *S. lycopersicum* was growing as a crop in a roadside field. Furthermore, I found individuals belonging to *S. pimpinellifolium* that looked purebred but had alleles that indicated they were crossing with *S. lycopersicum*. Throughout the literature there are examples *where S. pimpinellifolium* and *S. lycopersicum* have been merged as species as they are clearly closely related (Peralta *et al*., 2008 and references therein). It has been suggested that *S. pimpinellifolium* is the wild progenitor of the domestic tomato that is included in *S. lycopersicum* (S. Knapp, pers. comm.). On mainland South America wherever these two taxa grow in sympatry they form fertile hybrids (Peralta *et al*., 2008).

Hybridization between the endemic and the introduced taxa

Solanum cheesmaniae and *S. pimpinellifolium* hybrids

From a scientific point of view, finding hybrids between a local endemic and an introduced taxon in such an important plant as tomatoes is very exciting, but from a conservation point of view, taking the biology and natural history of tomatoes (and humans as their dispersal agents) into consideration, this is probably close to a nightmare scenario as far as the future of the endemic Galápagos tomatoes is concerned. I found clear evidence of hybridization and introgression – emanating from a rubbish dump on Santa Cruz, the most populated island.

A detailed field study was undertaken of the tomato populations towards the northern part of Isla Santa Cruz during 2000 and 2002. A tarmac road, the Baltra Road, dissects this part of Isla Santa Cruz. The Baltra Road runs from the most northern part of Santa Cruz, at the Canal de Itabaca (‘the Canal’)

towards the village of Santa Rosa in a south-westerly direction, continuing southeast to another village - Bella Vista. The road then goes due south to Puerto Ayora (Figure 3.11). It is the only road between the villages and the main town Puerto Ayora and the busy thoroughfare (via the Canal) to the national airport on Isla Baltra. This road is also the route to the island's current Basura (the rubbish tip 13.5km south west of the Canal) and two gravel mines – Mina Negra and Mina Roja (11km and 17km from the Canal). This Basura (rubbish tip), to the west of the Baltra road, was established in 1996 (Mark Gardener, pers. comm. 2008) after the Old Basura (just north of Puerto Ayora) was closed.

On Isla Santa Cruz, during this research, *S. cheesmaniae* accessions were only collected at lower elevations between sea level and c. 300m. *Solanum cheesmaniae* was collected as a coastal plant towards the north and northeastern part of the island (pops 101 -104 were collected in 2000 only). In 2000 *S. cheesmaniae* was not found further inland on Isla Santa Cruz. During field work in 2002 *S. cheesmaniae* accessions were found growing inland along the Baltra Road as a ruderal at the 'Zone of Birds' (pop 105) c. 6km inland, the Mina Negra (pop 106) 10km inland and as far south as the Basura (pop 107) at 300m altitude and (12km from the coast).

Conversely, the populations of *S. pimpinellifolium* from the northern parts of Isla Santa Cruz were collected from the moister regions, at higher altitudes, from between 600m and 300m altitude. *Solanum pimpinellifolium* accessions were also collected as ruderals along the Baltra Road the Basura area being the northern most point and further south at Mina Roja, at Los Gemelos and at El Chato (collected in all localities in both 2000 and 2002). *Solanum pimpinellifolium* was also found growing along the roadsides from Bella Vista towards Garapaterra Beach and towards Puerto Ayora as well as within Puerto Ayora itself.

During 2002 fieldwork the two species' ranges were found to overlap at the Basura and *S. cheesmaniae* x *S. pimpinellifolium* hybrids identified using morphological characters. *Solanum cheesmaniae* x *S. pimpinellifolium* hybrids were also collected at Mina Roja and to the north of the Basura at Mina Negra and the Zone of Birds.

Only at the Basura all three groups of tomatoes were found growing together - all within five meters of each other. In 2002 the Basura consisted of two long pits into which the towns' rubbish was tipped and burned. The extracted soil, which was removed to form the pits, was piled up into banks (c. 3m high) at the far end of the Basura. The accessions of *S. pimpinellifolium* were collected (2000 and 2002) from the top of these mounds of soil. Accessions of *S. cheesmaniae* were collected (2002) from the native scrub forest to the west of the Basura, while the *S. cheesmaniae* x *S. pimpinellifolium* hybrids were collected (2002) from between the two parent populations on sides of the banks of the mounds.

The center of genetic and morphological diversity for this group of tomatoes is at this Basura. One hypothesis might be that the Basura is the source of *S. pimpinellifolium* in this area or at least the Basura provides a disturbed habitat and a substrate into which the *S. pimpinellifolium* can colonize. Either the hybrids are radiating out from the Basura population or there are several independent areas along the Baltra Road where hybridization is taking place.

Hybridization was not established using morphology during fieldwork in 2000. Some allelic evidence, however, supports evidence of hybridization in these earlier collections both in *S. pimpinellifolium* along the Baltra road and in the north coast population of *S. cheesmaniae* (see Figure 3.11). There is some support in the allozyme data that levels of hybridization between *S. cheesmaniae* and *S. pimpinellifolium* might have increased between 2000 and 2002 (see DIA-2, 108 allele, Table 3.14), but these developments need to be explored over longer time periods.

Overall the Basura population had the highest N_e of all the hybrid populations, and overall the mean N_e for *S. cheesmaniae* (taken from the three hybrid zone populations Zone of Birds, Mina Negra and the Basura) was higher than the other taxa contradicting the results found for the *S. cheesmaniae* at the Basura. However all these N_e results are very low and very similar and perhaps too much should not be read into these.

Principal Coordinate Ordination Analysis (PCO) is an ordination method similar to PCA and is a useful method of interpretation of large multivariate data sets. PCO was undertaken as a method to interpret the morphological data of *S. cheesmaniae* and *S. pimpinellifolium* and to detect evidence of hybridization between these two species. PCO searches for similarities between cases and the set of coordinates that are produced puts these similar cases close to each other (http://uk.geocities.com/ahf_alternate/pco.htm; Dytham, 2003). PCO is commonly used for taxonomic studies (Spooner *et al.,* 1993) and it makes no assumption on groupings. It has clearly shown that the *S. cheesmaniae* x *S. pimpinellifolium* identified in the field are morphologically intermediate to the *S. cheesmaniae* and *S. pimpinellifolium* individuals. However, not all the taxa are perfectly separated. The pure *S. cheesmaniae* from El Lagoon de Manzanilla (Isla Isabela) and the pure *S. pimpinellifolium* from Garapaterra Road (Isla Santa Cruz) do not overlap on the graphs showing a morphological distinction. However, this is not complete in the three taxa found along the Baltra Road probably indicating that there is more hybridization than was initially identified. This can be seen by the percentage mixed offspring graphs too (Table 3.14; Figure 3.11).

As far as genetic studies were concerned the highest N_e = 1.1 was recorded from the hybrid populations of *S. cheesmaniae* x S*. pimpinellifolium* at the Basura in the Baltra road on Santa Cruz, with the N_e of *S. cheesmaniae* in the Basura population just below at 1.099 (Table 3.15, 3.16). This level of N_e was the highest found in the Galápagos tomatoes. This is not surprising since in a hybrid population one would expect admixture to be present and the Basura is the only population where all three taxa can be found growing together (*S. cheesmaniae*, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium*). Highest diversity on admixture, within species and on a continental scale were observed elsewhere. In European phylogeographic studies high genetic diversity may be an indicator of long-term population survival (Hewitt 1996), or may represent suture zones between recently-established populations sourced from multiple refugia: Petit *et al.* (2003) found the highest intrapopulation cpDNA haplotype diversity for 22 European tree and shrub species to the north of the Alps, in regions colonised postglacially. In *Fagus sylvatica,* high isozyme allelic richness was shown to correspond with glacial refugia identified from the

pollen record, but highest expected heterozygosity occurred in areas remote from these refugia (Comps *et al.* 2001).

Looking at the overall morphology, flowering and local ecology some speculation about the causes and consequences of this hybridization are opportune. Theoretically, flower morphology of *S. pimpinellifolium* with exserted stigmas and few individual flowers with included stigmas would promote outcrossing. However at the Basura population there were more individual plants of both *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* than there were *S. cheesmaniae*. In addition to this, *S. pimpinellifolium* plants are usually much bigger than *S. cheesmaniae* with many scrambling stems that can be up to 3m in length (see chapter 2 in the taxonomic treatment of *S. pimpinellifolium*). *Solanum pimpinellifolium* also had more inflorescences per plant than *S. cheesmaniae* (personal observation) although *S. pimpinellifolium* has fewer flowers per inflorescence than *S. cheesmaniae*. It is most likely that, all other factors being equal, that due to the higher numbers of flowers overall that *S. pimpinellifolium* or *S. cheesmaniae* x *S. pimpinellifolium* hybrids would predominantly be the pollen donors and *S. cheesmaniae* would be the recipient of the pollen because of a type of 'pollen swamping' by the more abundant *S. pimpinellifolium*.

In addition to this it was found that the *S. pimpinellifolium* plants had on average finished flowering before the *S. cheesmaniae (*Table 3.22). If this also indicated that the *S. cheesmaniae* plants started flowering later then perhaps many of the ripe fruit of *S. pimpinellifolium* may have been pollinated even before the *S. cheesmaniae* plants had started flowering. Difference between flowering times can make a big difference to the direction of pollen flow, particularly if the flowers continue to produce pollen after the stigma receptivity ceases. However, in cultivated tomatoes stigma receptivity lasts for five days (longer in cooler temperatures). That represents 1-2 days before anthesis and 2-3 days after anthesis (Scott and Angell, 1998). The stigma remains receptive until the flower wilts (Singh *et al*., 2005). No references are available for the stigma receptivity in Galápagos tomato flowers and not enough time was available in the course of this investigation to test this.

Individual accession analysed and offspring

The genetic diversity of accession 625 and its five offspring is interesting as it shows segregation or backcrossing and is a good indication of hybridization. In the field however this plant was described as *S. cheesmaniae* using morphological characters. It does however seem that it was probably an F1 hybrid, as the offspring (some or all of which may have been the result of self pollination) appear to have segregated/ backcrossed. The ratio here is ratio 1:2:2. Although this sample size is very small it is an interesting indication of hybridization.

Pollination and flower morphology

The solitary carpenter bee *Xylocopa darwini* is endemic to the Galápagos Islands and is the only native, and only recorded, bee in Galápagos. This is despite local interest in the introduction of honeybees. It has been suggested that the arrival to the islands of the ancestor to the *X. darwini* must have been quite late in the Galápagos Islands' history. The carpenter bee, as the name suggests, needs wood to make a nest and tree species are thought to be relatively late arrivals to oceanic islands – the herbaceous plants being the pioneer species. Although the presence of another earlier bee pollinator that is now extinct cannot be ruled out, it would seem that the Galápagos tomatoes probably evolved in the absence of a pollinator on the islands. It should also be noted that some islands on which tomatoes occur, for example Sombrero Chino, have no trees at all, so there is a strong need for these tomatoes to be self-sufficient as far as pollination is concerned – self-compatibility and inbreeding is the only route.

Bees are thought to be most active in the morning. Most bees only forage over a certain minimum temperature. In other areas *Xylocopa spp.* pollination habits have been observed in relation to temperature. For example the night foraging *X. tenuiscapa* only forages above a mean temperature of 7ºC whereas *X. capitata* will only forage above 23 ºC (Somanthan and Borges 2001). Within the genus therefore this is a wide range of minimum foraging temperatures but no specific references to *X. darwini*. During my fieldwork (2000 and 2002) I observed *X. darwini* bees buzz pollinating in several populations of tomatoes in

the field in Galápagos. I undertook bee observations on a population of *S. cheesmaniae* and *S. galapagense* at El Lagoon de Manzanilla near Puerto Villamil (Isla Isabela). Here I observed *X. darwini* buzz pollinating both species of endemic tomato. I also captured a bee and removed pollen from its corbiculae, which I later identified as *Solanum* pollen. Identification to species level was not possible although these tomatoes were the only *Solanum* spp. in the near vicinity.

To monitor bee activity I undertook a period of observation (at El Lagoon de Manzanilla, Isla Isabela) on a group of eight different tomato plants between 05.45-11.30 hours. Between 05.45 and 9.30hours there was no bee activity on these plants however the light rain or the low temperatures may have discouraged bees. Between 09.30 and 11.30 hours, the rain had stopped and bees visited 34 individual flowers belonging to eight different plants. The number of bees involved in these visits however was not recorded. These observations represent more bee activity than Knapp (1986) found during observations of other Solanaceae flowers, but also see Buchmann (1993) who recorded very high bee activity.

In common with many plant species flower morphology is one of the factors that determine whether a flower is self- pollinating or can be cross- pollinated. In the tomato flowers when the stigma is included within the staminal column only automatic self- pollination can take place (Rick, 1982) and cross- pollination is thus made less likely. If the stigma is exserted beyond the end of staminal column then cross-pollination is more likely. Rick *et al.* (1978) found that *S. pimpinellifolium* flowers where the stigma exsertion is over 1.5mm, self-pollination is precluded and the flowers cross-pollinate (see also Georgiady & Lord, 2002).

Throughout all the wild populations of *S. cheesmaniae* and *S. galapagense* in the Galápagos Islands there were very few individuals with exserted stigmas. Only two populations of pure Galápagos tomatoes were found in the field with over 25% of individuals with flowers with stigma exserted. These were both populations of *S. cheesmaniae* – one at NE of Caleta Webb (pop 307, Isla Isabela) where 92% (n=13) of the accessions had individual flowers with exserted stigmas and the other at Los Túneles (pop 606, Isla Fernandina)

where 67% (n=6) of the accessions had flowers with exserted stigmas. At the El Lagoon de Manzanilla (Isla Isabela), none of the *S. cheesmaniae* and *S. galapagense* had exserted stigmas. However, interestingly two out of the three *S.cheesmaniae* x *S. galapagense* hybrids in this population did have flowers with exserted stigmas.

The mixed populations of *S. cheesmaniae, S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* along the Baltra Road (Isla Santa Cruz) were found to have high levels of flowers with exserted stigmas in all three taxa only one individual plant in this population had a flower with an inserted stigma this was a *S. cheesmaniae.* A comparison between this population and *S. cheesmaniae* from El Lagoon de Manzanilla (Isla Isabela) and *S. pimpinellifolium* from Garapaterra Road (Isla Santa Cruz) shows a big difference (Figure 3.12).

These results on stigma exsertion however can only be viewed as an indication - only one flower per accession was measured due to time constraints. If this were repeated, at least five flowers per inflorescence and several inflorescences per plant should be measured. This would enable slightly more robust results. However this quantity of measurements was not possible during my fieldwork.

Stigma exsertion in tomatoes is controlled by different factors. For example genetic factors were found to control style length in *S. pimpinellifolium*. Heterostyly can also be affected by different temperature regimes. Plants of *S. lycopersicum* were grown in conditions with high day/night temperatures (35/24˚C) and stigma exsertion was promoted under these conditions This was compared to plants grown at lower temperatures (20/16˚C) where the flowers were found to have inserted stigmas (El-Abd and el-Beltagy, 1996). Fernandez-Muñoz and Cuartero (1991) reported similar results when measuring stigma exsertion in different *S. lycopersicum* cultivars. They found them to be highest under a high temperature regime but the results were only significant in some of the cultivars tested. Research with cultivated tomatoes demonstrated that stigmas can dry out in conditions of low humidity or windy weather and that this is detrimental to fruit set (Scott and Angell, 1998). Stigma insertion in

Galápagos tomatoes might help to prevent this drying out from taking place in this arid climate.

The staminal column of some Galápagos tomato flowers had red/brown markings (personal observation). This 'bruising' is most likely caused by the bees from buzz pollinating. Morandin *et al*. (2001) attributed similar sounding reported markings to bruising caused by bumble bees. They found that heavier bruising was a good indication of increased levels of bee visits and lead to increased stigmatic pollen loads. After the bees alight on a tomato flower, the weight of the *X. darwini* causes the flower to droop so that the bees hang upside down while clinging to the flower (personal observation). Further research in this area would be interesting. The evidence of pollinating bees is strong within the Galápagos tomatoes.

Despite the high level of hybridization found at the Basura population I never observed carpenter bees at this location during this research. However on several of the field days at this location the air was smoked filled due to rubbish burning. The presence of smoke, during certain wind conditions, might deter the bees.

Origins of the different tomatoes in Galápagos

Solanum lycopersicum and *S. pimpinellifolium* are thought to have diverged from a common ancestor about one million years ago and therefore the Galápagos tomatoes are thought to be of younger origin (Nuez *et al*., 2004). Rick and Fobes (1975) suggested that the progenitor of the Galápagos tomatoes was *S. pimpinellifolium* from the region of Motupe-Olmos of Dept. Lambayeque in Perú. Several reasons were put forward: 1) Populations of *S. pimpinellifolium* from the NW of Peru had the greatest similarity allozymatically to the Galápagos tomatoes, 2) Motupe-Olmos was also the area that *S. pimpinellifolium* plants were morphologically most similar to the Galápagos tomatoes, 3) The climate of this area is similar to the Galápagos climate, with occasional summer rains, 4) The plant species assemblage of this region which includes *Alternanthera*, *Parkinsonia* (both Fabaceae), *Tournefortia* (Boraginaceae) and *Waltheria* (Sterculiaceae) is very similar to the assemblage

of species that you might expect to find in a typical Galápagos tomato habitat, and finally 5) the Humboldt Current flows northwards along this part of the coast and then west towards the Galápagos Islands thus providing a potential route for the ancestors of Galápagos tomato plants, from the mainland of South America to the archipelago.

Nuez *et al*. (2004) report the occurrence of new plants in the highlands of Santa Cruz (from 2000) describing them as *Lycopersicon esculentum* 'Gal *cer*'. The 'Gal *cer*' is to reflect the smaller fruit at <1.5cm 'normal' *L. esculentum.* Darwin *et al*. (2003) found the fruit size of *S. pimpinellifolium* approximately the same size of under <1.6mm. Nuez *et al*. (2004) undertook AFLP analysis on their Galápagos tomato field collections, mainland Ecuador and Peru field collections and included some Galápagos accessions from the TGRC. In their analysis they reported four well supported clusters as follows: 1) all the endemic tomatoes together; 2) wild collected *S. pimpinellifolium* (as *L. pimpinellifolium*) from Peru and mainland Ecuador; 3) cultivated *S. lycopersicum* (as *L. esculentum*); 4) *L. esculentum* var. *cerasiforme* with *L. esculentum* 'Gal *cer'*.

This final cluster is made up of the plants that they describe as *Lycopersicon esculentum* 'Gal *cer'*, one individual that they describe as *L. esculentum* var. *esculentum* and two accessions from the TGRC which they refer to as *L.esculentum* var. *cerasiforme* (LA2856 – from Isla Isabela and LA3123- from Isla Santa Cruz). However these two accessions from the TGRC are (now) described by the TGRC as *S. pimpinellifolium.* This suggests that the Nuez *et al*. (2004) *Lycopersicon esculentum* 'Gal *cer'* is what I regard as *S. pimpinellifolium*. While I did not include TGRC LA2856 in this study, I did grow the accession LA3123 collected from the same locality. I considered this plant to correspond morphologically to *S. pimpinellifolium* collected from the same area near Los Gemelos on Isla Santa Cruz. Since publishing Darwin *et al*. (2003) and having been in correspondence the Roger Chetelat at the TGRC, the TGRC concurred and the name has been changed to *S. pimpinellifolium* for this accession (LA3123). A further complication with Nuez *et al*. (2004) dataset is that *S. pimpinellifolium* from South America might already be introgressed with *S. lycopersicum* (S.Knapp, personal observations). Nuez *et al*. (2004) report that that the *L. esculentum* 'Gal *cer*' had not been recorded before in the

Galápagos. However as *S. pimpinellifolium* it was first collected in the Islands by the TGRC from Isla Isabela in 1985 (LA2856), then also by the TGRC from Isla Santa Cruz 1991 (LA3123) and then by myself in 2000 (Darwin et al., 2003). Chapter 2 of this thesis reports the first unequivocal collections of *S. pimpinellifolium* and *S. lycopersicum* in the Galápagos Islands.

The question of when the first plants belonging to *S. pimpinellifolium* were introduced has been made all the more complicated due to the early descriptions of plants that are now called *S. cheesmaniae* having been described as *S. pimpinellifolium,* for example by Hooker (1847) and Rick and Bowman (1961). Some of the misnamed *S. pimpinellifolium* plants from the TGRC have only recently been changed (during the past ten years) on the TGRC web site. For example accession LA0166 (collected from Santa Cruz in 1950) was formerly named as *L. pimpinellifolium* (Rick and Bowman, 1961). It was not originally thought to be the introduced *S. pimpinellifolium* but named as such as the native biotype (R. Chetelat pers. comm.). For further information on the history of Galápagos tomato nomenclature see Darwin *et al*. (2003). However, the occurrence of *S. pimpinellifolium* on Isabela remains unconfirmed. I made one collection of a possible *S. pimpinellifolium* but it was too immature to be certain.

Threats and Extinction

The effect that hybridization between the rarer endemic tomatoes with the introduced tomatoes appears to be two fold. Firstly hybridization and introgression can lead to the rarer taxa becoming locally extinct due to introgression and genetic swamping and secondly the already invasive introduced tomato may become a more serious invasive problem due to individual plants acquiring local adaptations that may be beneficial and selectively advantageous.

Extinction of rare species is often blamed on environmental/demographic stochastic events or habitat change. This change may be abiotic or biotic for example increases in predation, disease or habitat change. Inbreeding depression or low levels of genetic variation may be contributing factors for extinction, although they are rarely more important than

environmental/demographic factors (Levin *et al*., 1996). Interspecific hybridization is rarely sited as a reason for rare plant species extinction. Extinction through hybridization takes place either by "demographic processes" or through assimilation by another species by introgression (Levin *et al*., 1996).

Demographic processes can cause extinctions of rare species by interrupting the population process of individual replacement. This would then affect the rate at which a population can grow (Levin *et al*., 1996). A reduction in the rate that population growth takes place can increase threats of extinction. Changes in population size are due to birth, growth and death rates. Growth rates of populations may be reduced by the production of hybrid seed regardless of whether the hybrid seed is viable or not. When hybridization is bidirectional a relatively small population can produce a higher percentage of hybrid seed than more "abundant congener" when intermixed. "the weaker the barriers the greater the minority disadvantage" (Levin *et al*., 1996).

Hybrids as invaders

Charles Darwin undertook plant breeding experiments and discovered that outcrossed offspring were often more vigorous (Darwin, 1876), the same can be found when plant taxa outcross with closely related taxa and the resultant offspring is more competitive than either parent. Invasive plant species are often found to have hybrid ancestry although evidence of this as a stimulus of invasiveness is not obvious (Rieseberg *et al*., 2007). Some morphological characters found in the *S. cheesmaniae* x *S. pimpinellifolium* hybrids show hybrid vigor; for example some flower size measurements were found to be larger than in either parent. Larger flowers are found to attract more bee visits (Somanthan and Borges, 2001). In addition, increased vigor of morphological characters genetic variation can increase in populations as a result of hybridization this provides "a larger pool of raw material for adaptive evolution" (Rieseberg *et al*., 2007 and references therein). Hybrids can have increased vigour as a result of increased levels of heterozygosity (Rieseberg *et al*., 2007). Invasiveness can be due to increased genetic variation alone and/or the input of "novel combinations of genes or phenotypes" (Rieseberg *et al*., 2007).

There are examples where the hybrid offspring can colonize new habitats and become an invasive problem. A classic example is *Spartina maritima* X *S. alternifolia* in the United Kingdom. *Spartina maritima* is a native salt marsh species in the UK and *S. alternifolia* is from the east coast of North America and was probably introduced accidentally from ship ballast water. This hybridization resulted ultimately in the now invasive *S. anglica* (Rieseberg *et al*., 2007). *Spartina anglica* causes problems in the UK salt marshes where it rapidly colonises mud flats causing habitat loss for waders and waterfowl and probably prevents colonisation by the native *S. maritima*. See Joint Nature Conservation Committee (JNCC) web site and references therein (http://www.jncc.gov.uk/page-1680).

The *S. cheesmaniae* x *S. pimpinellifolium* hybrid was also found to be intermediate in its ecology with a wider altitudinal range along the Baltra Road than either parent. *Solanum cheesmaniae* x *S. pimpinellifolium* occurred from the north at the Zone of Birds to the southern end of this stretch of road at Mina Roja. The acquisition of local adaptations resulting in range expansion has been suggested in the case of *Helianthus annuus* (Rieseberg *et al*., 2007).

The increased potential of *S. pimpinellifolium* becoming a more successful invader is a threat. It may be due to the addition of some locally acquired characteristics this species might become an even more serious invasive plant. This could effect not only the endemic tomatoes populations but also other taxa too. The Galápagos Islands are affected by extreme climatic conditions as well as the El Niño Southern Oscillations (ENSO) events. Native/endemic plant species including the tomatoes in Galápagos have evolved to cope with natural climatic change however it is possible that these periods of extreme climatic conditions may be the testing time for the invasive species. The last major El Niño event started in 1997 and caused high levels of rainfall in 1998 that was beneficial for many of the plant species. *Solanum pimpinellifolium* would probably benefit from an increase in rainfall; it doesn't seem to cope so well in the arid areas in the Galápagos (http://www.pbs.org/safarchive/5_cool/Galápagos/g24_weather.html and personal observations). The creation of the rubbish dump, known here as the Basura, on Santa Cruz (1996) was shortly before the beginning of the most

recent El Niño and this may have been a contributory factor in the movement of *S. pimpinellifolium* from Los Gemelos where we know it occurred in 1991 (TGRC LA3123) further north.

Most domestic plants are found to cross breed with their wild relatives. The gene flow from crop plant to wild relatives may have a significant impact on the evolution of the wild plants (Ellstrand *et al*., 1999). Rubbish dumps in open areas are known to provide a substrate for ruderal species, invasives and agricultural weeds. It is also suggested that rubbish dumps can contribute to the spread of invasive plants however there seems to be very little published on this subject (Pysek, *et al*., 2003). Pysek *et al*. (2003) studied plant species diversity in Czech Republic rubbish dumps. Human population density and species richness in rubbish dumps was positively correlated. A study of disturbed sites on Isla Santa Cruz has been undertaken by the Parque National Galápagos. Higher levels of invasive species have been found in the disturbed sites of the rubbish dump on the Baltra Road and the gravel mines. In addition to this invasive birds found in these disturbed sites may also be responsible for seed dispersal, especially introduced plants. This may include seed dispersal into nearby and undisturbed sites. (http://www.hear.org/Galápagos/invasives/spotlight/index.html).

Within recent history rubbish dumps are clearly important locations for plant evolution. It has long been suggested, for example, that some crop plants evolved in rubbish dumps due to hybridization. Sometimes called “treasure in the trash syndrome” (see http://www.physorg.com/news106838117.html)

Chapter 3c

Genetics of tomatoes in seed banks

Introduction to seed banks and the Tomato Genetic Resource Center (TGRC)

The purpose of seed banks is to preserve seeds of wild plants or cultivated lines of crop and other useful plants and their close relatives for future generations of plant breeders. Wild crop plants may have characters that can be useful to breed into crop plants - for example resistance to diseases, medicinal or nutritional benefits, or morphological traits.

Seed banks representing all major crop plants have been set up mainly by public organisations or large agricultural businesses in different areas around the world to store and preserve wild relatives and cultivated lines of crop plants. The main seed bank collection of tomatoes is stored at the Tomato Genetic Resource Center (TGRC) at UC Davis, Davis, California. Seeds of hundreds of accessions of tomatoes and their wild relatives are stored here and made freely available for research and breeding programmes (TGRC website: http://tgrc.ucdavis.edu/).

Each collection of seeds at the TGRC is called an accession, which represents a collection of seeds harvested originally from wild tomatoes, in this case, from the Galápagos Islands. Each accession was collected from a single species at a single locality, but seeds may have been harvested from a number of plants within close proximity. Detailed information is given for each accession – they are is given a unique 'LA' number (standing for *Lycopersicon* accession, in the case of the TGRC), date of collection, location and other information on a document that is known as a passport. These seeds are stored at the optimum temperature and humidity for each species. Every few years the seeds undergo rejuvenation cycles to ensure viability. During these rejuvenation cycles the plants are grown up in the field and seeds from the offspring are then harvested and stored again until the next cycle (TGRC website: http://tgrc.ucdavis.edu/).

Accessions of Galápagos tomatoes sourced from two seed banks during this research – from the TGRC and from a collection held at Cornell University. The tomato seeds stored at Cornell University were originally TGRC seed bank accessions so their LA numbers are included. These seed bank accessions were also analysed and compared to the field-collected material from similar localities.

The questions raised here were to investigate if there is a difference between the seed bank accessions and the SCD field collections with the allozyme electrophoresis and to see if there is evidence of hybridization within the seed bank accessions.

Materials and Methods

Accessions

Altogether 60 seed bank accessions from tomato plants originally collected from Galápagos Islands were analysed for allozyme electrophoresis. I used on average 4.1 plants per accession. This represented 6 accessions from the Cornell University seed bank and 54 accessions from the TGRC. Seed dormancy and growing conditions were consistent with those outlined in the general Material and Methods above. The seed bank accessions represented 37 *S. cheesmaniae* accessions, 21 *S. galapagense* accessions, one *S. pimpinellifolium* accession and one *S. lycopersicum* accession from altogether 11 different main islands (excluding the islets of the main islands). Tables 3.23.1 and 3.23.2 presents the details of each accession from the seed banks.

Table 3.23.1. List of accessions from Cornell seed bank used for the study of allozyme electrophoresis

TGRC acc. No.	SCD acc no.	n=	Coll. Date	Island name	Locality name
S. cheesmaniae					
LA1406	003	5	1970	Fernandina	Crater (S/W rim caldera)
LA1404	002	5	1977	Fernandina	Crater (west flank caldera)
LA1412	005	7	1971	San Cristóbal	Opp.Isla Lobos
S.galapagense					
LA1403	006	8	1971	Fernandina	West of Punta Espinoza
LA1410	001	11	1971	Isabela	Punta Ecuador (coast)
LA1411	004	8	1971	Santiago	James Bay

Table 3.23.2. List of accessions from TGRC seed bank used for the study of allozyme electrophoresis

TGRC acc. No.	SCD acc no.	n	Coll. Date	Island name	Locality name
S. cheesmaniae					
LA0531	022	6	1956	Baltra	North Coast
LA1427	038	2	1971	Fernandina	Crater Rim (W/S-W)
LA0529	042	4	1957	Fernandina	Edge of crater lake
LA0522	043	3	19	Fernandina	Crater (outer slopes 1000m)
LA0521	044	4	1958	Fernandina	Crater (inside)
LA1402	039	3	1971	Fernandina	Punta Espinoza (W)
LA1035	040	5	1964	Fernandina	Low Elevations SE side
LA0749	041	3	1960	Fernandina	North Side
LA1042	026	4	1965	Isabela	Cerro San Tomás
LA0437	036	4	1956	Isabela	Villamil (ponds to the north)
LA1043	037	2	1966	Isabela	Cerro San Tomás
LA1139	029	4	1968	Isabela	E Cerro Azul
LA1138	030	3	1968	Isabela	E Cerro Azul
LA1037	031	1	1965	Isabela	Alcedo crater bottom
LA0746	034	7	1960	Isabela	Punta Essex (1km inland)
LA0524	035	2	1960	Isabela	Punta Essex (100m inland)
LA1409	028	3	1971	Isabela	Punta Albemarle (3km west)
LA1036	032	4	1964	Isabela	Far North
LA0932	033	3	1964	Isabela	Tagus Cove
LA1039	053	3	1965	Isabela	Cape Berkley
LA0422	024	1	1956	San Cristóbal	Wreck Bay
LA0421	025	3	1956	San Cristóbal	Wreck Bay
LA1040	023	4	1956	San Cristóbal	Caleta Tortuga
LA0429	016	4	1956	Santa Cruz	Los Gemelos
LA1041	017	7	1965	Santa Cruz	El Cascajo
LA0428	019	9	1956	Santa Cruz	Puerto Ayora to Bella Vista trail
LA1449	013	8	1971	Santa Cruz	CDRS
LA0927	014	4	1964	Santa Cruz	Puerto Ayora
LA0528B	015	8	1957	Santa Cruz	Puerto Ayora
LA1447	018	4	1971	Santa Cruz	Punta Nuñez
LA1448	020	2	1971	Santa Cruz	Pelican Bay [Puerto Ayora]
LA0434	021	3	1956	Santa Cruz	Nr Puerto Ayora Rambech's trail
LA0166	077	2	1950	Santa Cruz	Barranco North of Punta Ayora
LA3124	045	4	1991	Santa Fe	East Landing

Table 3.23.2. continued

TGRC acc. No.	SCD acc no.	n	Coll. Date	Island name	Locality name
S. galapagense					
LA0530	059	4	1957	Fernandina	Edge of crater lake
LA0483	060	4	1956	Fernandina	Crater inside
LA1508	066	2	1972	Floreana	Corona del Diablo
LA1136	065	4	1967	Floreana	Gardner
LA0438	027	3	1956	Isabela	Villamil (coast)
LA0436	056	2	1956	Isabela	Villamil
LA1452	057	3	1969	Isabela	Alcedo (East Side 350m)
LA480A	051	5	1957	Isabela	Cowley Bay
LA1400	052	4	1971	Isabela	Playa Tortuga Negra etc
LA0930	054	1	1961	Isabela	Playa Tortuga Negra etc
LA0929	055	3	1962	Isabela	Playa Tortuga Negra etc
LA0532	064	2	1958	Pinzón	North West side
LA1137	062	2	1967	Rábida	North Side
LA1044	048	2	1966	Santiago	Bartolomé
LA0527	049	4	1957	Santiago	Bartolomé
LA0426	050	3	1956	Santiago	Bartolomé
LA0474	047	4	1960	Santiago	Cape Trenton
LA0748	063	3	1960	Santiago	Trenton Island (E)
S. pimpinellifolium					
LA3123	069	6	1991	Santa Cruz	Towards Baltra
S. lycopersicum					
LA0292	067	9	1952	Santa Cruz	Playa to Bella Vista trail

Presence tables

Presence tables were created to show the maximum allelic diversity found in the seed bank accessions, an overall allele presence table was also created to show the overall allelic diversity found within the seed bank and the wild-collected accessions from the Galápagos Islands.

Within-population genetic diversity

Within-population genetic diversity measures were calculated represented in tables 3.26.1-5 representing each species and the different islands. For further details, please see my general materials and methods section.

F-statistics

F-statistics were calculated for each of the species found in the seed banks and these were represented in Tables 3.27 and 3.28. For further details, please see my general materials and methods section.

Results

Allele presence tables

Presence tables (Tables 3.24, 3.25) were constructed to show the overall diversity in all the Galápagos tomatoes as a comparison with the seed bank accessions. A presence table (Table 3.24) illustrates diversity for the seed bank accessions. Overall the results show that there are more alleles found in the seed bank accessions than in the wild collections. This even includes one allele in an otherwise invariable locus, UGPP (Table 3.24).

Tables 3.26 give the results for the within-population genetic diversity measures for the seed bank accessions. Table 3.26.1 shows an overall summary of all the taxa. The results show that, overall and in comparison with other plants species, there are very low levels of diversity found within the seed bank accessions (see Hamrick & Godt, 1990). Subtle differences show that *S. galapagense* with a N_e of 1.03 has the most diversity, followed by *S. cheesmaniae* at 1.008. *Solanum pimpinellifolium* and *S. lycopersicum* were found to have no diversity at all, but this is only based on a single accession each.

A comparison between the seed bank accessions and the SCD accessions show that the seed bank *S. galapagense* N_e is higher than the SCD collection for *S. galapagense*. The seed bank and the SCD collections had nearly the same N_e in *S. cheesmaniae* but again the seed bank had more allelic diversity that I recovered from the much more extensive field collections.

Table 3.26.2 shows the mean diversity measures for *S. cheesmaniae* on all the different islands. Here Isla Baltra was found to be the most and Isabela the least diverse. In *S. galapagense*, on the other hand, Isabela was found to be the most diverse, and several islands in *S. galapagense* had no diversity at all (see table 3.26.3).

Table 3.24. Summary of allele presence in all four taxa of Galápagos tomatoes (SCD and seed bank collections).

Locus	allele	*S. cheesmaniae*	*S. galapagense*	*S. pimpinellifolium*	*S. lycopersicum*
PGI-2	160		160		
	145	(145) *	145		
	130		130		
	121	(121) *			
	100	100	100	100	100
UGPP-2	112	(112) *			
	100	100	100	100	100
PGM-1	106	(106) *	(106) #	106	
	100	100	100	100	100
PGM-2	129				
	113	113	113	(113) #	
	100	(100) *&	(100) *#	100	100
MDH-3	104		104		
	100	100	(100) * #	100	100
	79	79*			
IDH-1	100	100	100	100	100
	95	95			
6-PGD-2	114	114*			
	103		103		
	100	100	100	100	100
DIA-1	110				
	100	100	100	100	100
	85		85		
DIA-2	108	10	108	(108) #	108
	100	(100)#	(100)#	100	(100) #
DIA-3	132	132 #		(132)#	
	100	100	100	100	100

Legend

Taxa	**Colour code**
All taxa	
S. cheesmaniae and S. galapagense	
S. cheesmaniae	
S. galapagense	
S. pimpinellifolium	
S. cheesmaniae, S. galapagense, S. lycopersicum	

Bracketed alleles indicate putative hybrids

* indicates seed bank accessions

# indicates wild accessions which are sympatric with other taxa

& indicates wild accessions which are **not** sympatric with other taxa

Table 3.25. Allele presence in TGRC and Cornell seed bank collections. This was calculated using the bulk data set to show the maximum level of allelic diversity.

		S. cheesmaniae							*S.galapagense*							*S. pimp*		*S. lyc*
Locus	allele	Baltra	Santa Cruz	Santa Fé	Isabela	Fernandina	San Cristóbal		Isabela	Floreana	Rábida	Fernandina	Pinzón	Santiago		Santa Cruz		Santa Cruz
PGI-2																		
	160																	
	145																	
	130																	
	121																	
	100																	
UGPP-2	112																	
	100																	
PGM-1	106																	
	100																	
PGM-2	129																	
	113																	
	100																	
MDH-3	104																	
	100																	
	79																	
6-PGD-2	114																	
	103																	
	100																	
IDH-1	100																	
	95																	
DIA-1	110																	
	100																	
	85																	
DIA-2	115																	
	108																	
	100																	
DIA-3	132																	
	100																	

Legend (previous page)

Taxa	Colour code
S. cheesmaniae	
S. galapagense	
S. cheesmaniae x S. galapagense	
S. pimpinellifolium	
S. lycopersicum	
S. pimpinellifolium x S. lycopersicum	

Within-population genetic diversity measures

Table 3.26.1. Within population genetic diversity summary measures for all taxa (seed bank bulk)

Species	no.acc	n	N_a	N_e	H_O	H_e	Nei
S. cheesmaniae	30	150	1.027	1.008	0.00101	0.006	0.006
S. galapagense	19	82	1.045	1.031	0.00183	0.019	0.017
S. pimpinellifolium	1	6	1	1	0	0	0
S. lycopersicum	1	9	1	1	0	0	0
Mean	**51**	**247**	**1.75**	**1.103**	**0.0022**	**0.071**	**0.071**

Table 3.26.2. Within population genetic diversity measures for *S. cheesmaniae* (seed bank bulk)

Pop name	Pop no.	no.acc	n	N_a	N_e	H_O	H_e	Nei
Baltra	0	Total 1	6	1.1	1.039	0	0.03	0.028
Santa Cruz Mean	1	Total 6	57	1.038	1.01	0.00035	0.007	0.007
Santa Fé	2	Total 1	4	1	1	0	0	0
Isabela Mean	3	Total 12	40	1.006	1.006	0	0.003	0.003
Fernandina Mean	6	Total 7	34	1.05	1.009	0.00273	0.008	0.008
San Cristóbal Mean	9	Total 4	15	1.021	1.003	0.00297	0.003	0.003
***S. cheesmaniae* Mean**		**31**	**156**	**1.027**	**1.008**	**0.00101**	**0.006**	**0.006**

Table 3.26.3. Within-population genetic diversity measures for *S. galapagense* (seed bank bulk)

Pop name	Pop no.	no.acc	n	N_a	N_e	H_O	H_e	Nei
Isabela Mean	3	Total 6	32	1.082	1.0549	0.00548	0.0368	0.033
Floreana Mean	4	Total 2	6	1	1	0	0	0
Rabida Mean	5	Total 1	2	1	1	0	0	0
Fernandina Mean	6	Total 3	16	1.029	1.0177	0	0.0118	0.011
Pinzón Mean	7	Total 1	2	1	1	0	0	0
Santiago Mean	8	Total 6	24	1.052	1.0379	0	0.019	0.018
***S. galapagense* mean**	*/*	**19**	**82**	**1.045**	**1.0307**	**0.00183**	**0.0189**	**0.017**

Table 3.26.4. Within population genetic diversity measures for *S. pimpinellifolium (*seed bank bulk)

Pop name	Pop no.	no.acc	n	N_a	N_e	H_O	H_e	Nei
Santa Cruz	1	1	6	1	1	0	0	0
***S. pimpinellifolium* Mean**	**1**	**1**	**6**	**1**	**1**	**0**	**0**	**0**

Table 3.26.5. Within population genetic diversity measures for *S. lycopersicum* (seed bank bulk)

Pop name	Pop no.	no.acc	n	N_a	N_e	H_O	H_e	Nei
Santa Cruz	*/*	**1**	**9**	**1**	**1**	**0**	**0**	**0**
***S. lycopersicum* Mean**	*/*	**1**	**9**	**1**	**1**	**0**	**0**	**0**

F-statistics

F- statistics were undertaken in seed bank (both individual and bulk). All taxa are found to have very high measures showing extreme departure from Hardy-Weinberg Equilibrium (Table 3.27). The bulk and the individual group results are very similar to each other.

The highest F_{is} (greatest departure from H-W equilibrium) was found in the seed bank bulk collection where it is just under 1 showing that these 'populations' are almost entirely inbreeding. A lower F_{is} was recovered from the SCD individual collection. The F-statistics for *S. cheesmaniae* and *S. galapagense* for the seed bank accessions are displayed in Table 3.28 where the F_{is} is much higher in *S. galapagense* but the F_{st} is about the same for both taxa. The mean F_{st} is about the same in both the seed bank and the SCD collection.

Table 3.27 shows the F-statistics for TGRC and includes the SCD bulk and individual as a comparison.

Table 3.27. Summary of the F-statistics for all test groups (all taxa for each group)

Test group	F_{is}	F_{it}	F_{st}
SCD Bulk	0.5382	0.9286	0.8453
SCD individual	0.5126	0.9367	0.8701
TGRC Bulk	0.8811	0.988	0.8991
TGRC individual	0.8867	0.9945	0.9513

Table 3.28 Summary of the F-statistics 'pure' *S. cheesmaniae* and *S. galapagense*

Test group	Sample Size (no.plants)	F_{is}	F_{it}	F_{st}
Che Mean	150	0.6701	0.9246	0.7716
Gal Mean	82	0.9010	0.9731	0.7284
Overall Mean	232	0.8540	0.9814	0.8725

Neutrality tests

The neutrality test (as in Chapter 3a) shows that there was no evidence for selection on genetic variation in these seed bank accessions.

SCD field accessions and seed bank accessions – a comparison

Several alleles were found within seed bank accessions that were either not found at all in wild-collected SCD tomatoes or were not found in the relevant taxa within wild accessions. Some of this variation may represent alleles that were not found in plants collected during my fieldwork – *S. cheesmaniae* was not collected or found by SCD from Isla San Cristóbal during this fieldwork. The TGRC collections of *S. cheesmaniae* from Isla San Cristóbal show a private allele. However of the six Cornell seed bank accessions, three of them (one S. cheesmaniae and two S. galapagense) were found to have alleles in that were only found in *S. pimpinellifolium* or hybrids thereof in the SCD wild collections. Please see table 3.29 for further details.

Examples of apparent discrepancies between the diversity results are shown in Tables 3.24, 3.25 & 3.29 in order to explain the emerging picture of higher allelic diversity in the seed bank collections in comparison to wild collections. The DIA-1-85 allele was found in older TGRC collections (before 1971) from the west coast of Isabela but is now absent in any recent collection of TGRC and SCD from this area. However it is still found on the south, north and east coast on Isabela as well as in accessions that were collected from several other sites throughout the archipelago – Islas Pinzón, Bartolomé and Rabída. DIA-1-85 was not found in any accessions from Fernandina – either SCD or TGRC and although it was found in SCD from Pinzón, it was not found in the TGRC accession from Pinzón. There have been seven volcanic eruptions for example on Isla Fernandina between 1958 and 1998 and on Isabela, Volcán Cerro Azul erupted in 1979 and 1998 (Stevenson, 2000). Plants with this allele may have been wiped out during one of these volcanic events and due to founder effect the re-colonising populations lacking this allele. Table 3.29 shows all the allelic variation between the Cornell seed bank, the TGRC seed bank and the SCD accessions.

Recent lava flows have occurred along the NE and NW flanks of Volcán Cerro Azul (http://www.volcano.si.edu/world/volcano.cfm?vnum=1503-06=) on Isla Isabella. This area might include the area where the tomatoes are found along the coast, e.g. at Punta Tortuga. Absence of the DIA-1-85 in several

populations from localities that previously contained this rarer allele could be due to sampling error during this study.

In the wild-collected accessions of *S. galapagense* PGI -2 100 was not found in any of the Isla Isabela west coast populations, nor on Fernandina but again in the seed bank populations (LA1400) the PGI-2 130 allele is present and LA930 the PGI-2 145 allele were present. It seems that this was either due to sampling error or that the seed bank accessions include plants possessing some of the rarer alleles that have not been found in the wild collections made during this study. This could be due to chance and sampling error or it could be that these alleles have become extinct, perhaps due to recent lava flows from volcanic eruptions and/or grazing in the area by goats. It is possible that these areas have been re-colonised by populations which lack these rarer alleles for example from the nearby Isla Fernandina which appear to lack this rarer alleles both in all the tested seed bank accessions and also in the more recently collected samples made during this study.

The 6-PGD-2 114 allele in *S. cheesmaniae* occurs only in the seed bank accessions from two islands. It seems likely that this represents real diversity (rather than contamination). SCD never collected *S. cheesmaniae* on Isla San Cristóbal and had only two accessions from Isla Santa Fé from different localities to the TGRC accession from this island.

Table 3.29. Allelic anomalies between the Cornell seed bank, the TGRC seed bank and SCD accessions with a suggestion as to the cause.

Acc. Source ⇨	**Cornell**	**Cornell**	**Cornell**	**Cornell**	**Cornell**	**TGRC**	**SCD**	**Comment**
Accession ⇨ **Allele ⇩**	**LA 1410 (SCD 001) Punta Ecuador Isabela *S. gal***	**LA1404 (SCD 002) Fernandina crater *S. che***	**LA1411 (SCD 004) Santiago *S. gal***	**LA1412 *S.* San Cristobal *S. che***	**LA1403 W. punta Espinosa, Fernandiana *S. gal***			
PGI-2-121		100/100 100/121 121/121						Either private to this acc. or represents a seed bank hybrid with another acc.or taxa
PGI-2-145				X				Either private to this acc.or seed bank hybrid with another acc. or taxa
UGPP-2-112		100/100 100/112 112/112						Seed bank hybridization with another acc. or taxa
PGM-1-106		100/100 100/106 106/106					*S.pimp*, *S.che x S.pimp* hybrids	Seed bank hybridization with *S. pimp*
PGM-2-100	X		X			TGRC LA3124 Santa Fé *S.che*	*S.pimp* *S.che* x *S.pimp* hybrids Pinzon *S.che* *S. lyc*	Seed bank hybridization with *S.pimp* and or hybridisation in the islands with *S. pimp* or *S. lyc* prior to collection.
MDH-3-100					X		*S.che*, *S.che x S.gal* hybrids	Field hybrid with *S. che*
6-PGD- 114						TGRC Wreck Bay San Cristóbal LA0422, LA0421	/	Private allele found only in Wreck Bay, San Cristóbal not collected by SCD
IDH-100						North coast Baltra	Not N. coast Baltra, but in most other pops, in all other taxa	Natural allelic variation found in wild
DIA-2-100						LA1449 CDRS *S.che* LA1042,LA1043 San Tomas, Isabela *S.che*	SCD 206 Baltra *S.che* (one plant) SCD 223 North Coast, Santa Cruz (one plant) SCD 567 El Lagoon, Isabela, *S.gal* *S. pimp* Santa Cruz hybrids	Allele private to *S. pimp* so either due to field hybridization *with S. pimp* and/or hybridization with *S. pimp* in the seed bank
DIA-3-132						LA0531 N. coast Baltra *S.che*, LA0746 Punta Essex Isabela *S.che*	Mirador *S. che*	Seed bank hybridization with *S. pimp*

Legend: (previous page)

S. che	=	*S. cheesmaniae*
S. gal	=	*S. galapagense*
S. pimp	=	*S. pimpinellifolium*
S. lyc	=	*S. lycopersicum*
Acc.	=	Accession

Discussion

There was a considerable difference in the allelic diversity when comparing wild collections, representing some 700 accessions with the 60 or so seed bank accessions. The smaller sample size from seed bank showed greater allelic diversity than the c. 10x larger field collections. In the seed bank collections, new and different alleles were found within the seed bank accessions compared to the SCD wild collected accessions. Some of these allelic differences represented wild alleles that were almost certainly private to populations that were not collected during this research, e.g. *S. cheesmaniae* collections from Isla San Cristóbal. Because no populations of *S. cheesmaniae* were collected from Isla San Cristóbal during this research thus it is not possible to assess whether this diversity is a result of natural variation in the wild or hybridization in the seed bank. However two of the Cornell University accessions of *S. galapagense* (LA1410 and LA1411 from Islas Isabela and Santiago respectively) had alleles that were otherwise private to either *S. pimpinellifolium* or *S. lycopersicum* (PGM-2-100) during this research. One further Cornell accession of *S. cheesmaniae* from Isla Fernandina (LA1404) also had an allele that was only found in *S. pimpinellifolium* (PGM-1-106). Of particular interest were some of the accessions from Cornell University. Only six accessions were grown from Cornell seed bank four and several of these have different alleles from SCD collections (Table 3.29).

Intrapopulation allozyme diversity indices for Galápagos tomatoes

F statistics

All groups tested had very high F_{is} which shows a high degree of deviation from Hardy-Weinberg equilibrium and indicating that the populations are either inbreeding and/or that the plants are largely self-fertilised or suffering from genetic drift. Rick (1963) wrote that the endemic Galápagos tomatoes were largely autogamous. Self-fertilisation is the most extreme form of non-random mating (Lowe *et al*., 2004) and inbreeding. Small population sizes leading to genetic drift is also possible.

The highest mean of F_{is} = 0.88 was found in the seed bank test group. This could be an indication of fewer founders forming the basis of these seed bank accessions and some 'populations', for the sake of this study, arose from a single accession. Seed banks tests their seed accessions for germinability and when the germination level falls below a certain threshold in an individual accession, it is then grown up and the seeds are harvested and stored. The result of these seed bank rejuvenation cycles can cause inbreeding and the possibility of a gradual erosion of the rarer alleles by chance. Some of the accessions were collected from the Galápagos Islands in the 1950s for example *S. cheesmsaniae* LA 0429 (Isla Santa Cruz, 1956). The accessions undergo regular rejuvenation cycles at TGRC.

The F_{st} is very high in all test groups, particularly high in the non-species test groups (i.e. bulk, individual for the SCD and seed bank collections). This shows that there is more genetic diversity between the populations than there is within the populations. In the non-species test groups part of this can be attributed to the differences between the taxa with the biggest differences between the endemic and the introduced exacerbating this. The F_{st} is still very high in the species test groups.

Population analysis in between the Cornell and TGRC seed banks and SCD accessions

The seed bank accessions were analyzed separately. Then overall allelic diversity of Cornell and TGRC accessions was compared to the SCD accessions. A detailed assessment was undertaken to compare the alleles that were unique to the TGRC and Cornell seed banks' accessions. More alleles were found within the seed bank accessions than in the SCD collected accessions. For example the TGRC accessions of *S. cheesmaniae* have a total of 22 different alleles; this is compared to only 16 alleles in the SCD accession of *S. cheesmaniae.* The higher number of alleles that were found in the TGRC and Cornell compared to the SCD accessions could be attributed to several different scenarios: 1) private and unique alleles were not collected during the SCD field trips – 2000 and 2002; 2) populations with these unique alleles that were collected on behalf of TGRC and Cornell are now extinct in the wild; 3) hybridization between other accessions of *Solanum spp*. within the seed banks during a rejuvenation cycle; 4) hybridization in the wild and private alleles to accessions collected from unique areas. The Cornell seed bank accessions came originally from the TGRC seed bank. These appeared to be particularly diverse. Five of the six Cornell accessions (SCD 001-006) grown during this research contain alleles either not found during this research in either SCD accessions or other TGRC accessions or only found in SCD *S. lycopersicum*, *S. pimpinellifolium*, or *S. cheesmaniae* x *S. pimpinellifolium* hybrids from Baltra Road populations. One Cornell accession (LA1404) had two alleles in two different loci that were not found in any accession in this study and a third allele that, during this research, was only found in *S. pimpinellifolium*, *S. lycopersicum* and *S. cheesmaniae* x *S. pimpinellifolium*. Although most seed bank accessions have no observed heterozygosity, in this one Cornell seed bank accession (LA1404) there were both homozygotes and heterozygotes within different individuals grown from each accession for all three of these alleles in the three different loci.

Considerable funds have been used to establish seed banks both for conserving wild plant species (for example the Millennium Seed Bank at RBG Kew: http://www.kew.org/msbp/index.htm) and crop landraces as well as wild crop relatives (for example the TGRC). Much research has also been

undertaken to maximise genetic diversity at the point of collection for *ex situ* seed bank collections (e.g., Brown and Briggs, 1991). However, little research has been undertaken to ensure that the genetic diversity is conserved within the seed bank (Parzies *et al*., 2000). Safe storage of seeds in seed banks involves regular rejuvenation cycles to ensure continued seed viability. In the case of barley (*Hordeum vulgare* subsp. *vulgare*) rejuvenation takes place on average about every five years. Parzies *et al*. (2000) examined seed bank accessions of barley to establish if there was a correlation between the length of accession storage time and loss of genetic variation. Their results showed that genetic loss was associated with increased lengths of time that the accessions had been housed in the seed bank. The longer an accession had been stored in the *ex situ* seed bank the less gene diversity, alleles per locus and polymorphic loci the accessions had due to genetic drift (Parzies *et al*., 2000).

The increased levels of genetic diversity observed here, particularly within the Cornell seed bank, would thus seem counterintuitive. One would expect genetic drift to take place causing a reduction of diversity. Heterozygotes are rare within the Galápagos tomatoes anyway so the presence of heterozygotes in the Cornell seed bank *S. cheesmaniae* from Fernandina (LA1404) probably indicates hybridization within the seed bank. *Solanum cheesmaniae* and *S. galapagense* are both described by the TGRC as inbreeding and self-pollinating. One can however speculate whether sufficient precautions are being taken during the rejuvenation cycles to safeguard tomatoes from crossbreeding. Following this up would be an interesting study in its own right, looking at a number of different taxa in seed banks with rejuvenation cycles and comparing these data with the same species and their diversity in the wild.

Nakazato *et al*. (2008) assume that germplasm stored in seed banks would change (or alleviate) the selective pressures that were originally experienced by the plants in their wild habitats. Nakazato *et al*. (2008) undertook an assessment to compare collection with genetic variation in some TGRC accessions and found that there was no association between the genetic diversity and the time past since the original collection.

Many studies that use seed bank accessions just rely on their identification and take it for grated that no contamination of the material has taken place. Furthermore, many studies seem not to compare large numbers of wild collected accessions with seed bank accessions and a good example here is the Galápagos tomato study by Nuez *et al*. (2004), which included material from all over the archipelago and seed bank accessions, but was not critical about their material. An analogous situation exists with the widespread but uncritical use of Genbank sequences where users of these data assume that the sequence belongs to a specific species and include them as such into their phylogenetic or evolutionary studies. However, Bridge *et al.* (2003) demonstrated that up to 50% of all DNA sequences of fungi deposited in GenBank may be attributed to the wrong organism. There seem to be common problems with the taxonomy, identification and authenticity in public access databases and repositories, potentially including material in seed banks.

I found a slight difference in germination time between the wild collected and the seed bank accessions. The seed bank accessions germinated slightly faster, on average three days (and after the initial treatment), than the wild-collected accessions. This suggested that there might have already been some selection towards plants that germinate quickly after TGRC seed dormancy treatment (see General Materials and Methods, above). Given these artificial selection pressures one might expect that some of the traits that are found in wild, for example drought tolerance, might eventually be selected against and ultimately be lost in seed bank. It is also unlikely that exact natural conditions can be mimicked during rejuvenation cycles. The effects on genetic, physiological and morphological characters in plants that have been held in seed banks for long periods of time would be an interesting area to research.

Chapter 4

General discussion and conclusions

Species on islands, their morphological and genetic diversity, evolution, origins, taxonomy, systematics and conservation are the subjects of a dynamic and ongoing field of scientific endeavour. Crawford *et al*. (2006) demonstrated that there are often low levels of genetic diversity in island species and he used *Tolpis* spp. (Asteraceae) from the Canary Islands as a prime example. While island species of *Tolpis* can often be morphologically diverse, allozyme electrophoresis between island and congeneric mainland species revealed less variation in island taxa. Crawford *et al*. (2006) explained that this is due to the relatively short time frames for intraspecific divergence and accumulation of mutations in island populations in comparison with continental groups, especially when neutral or near neutral allozyme markers are concerned. In addition to this Crawford *et al*. (2006, and references therein) reported that allozyme diversity often does not correspond to morphological taxonomic species delimitations. However, in my study on Galápagos tomatoes, despite discovering very low levels of diversity, I was able to use allozyme diversity as diagnostic markers to delimit species and these delimitations were congruent with morphological evidence (Darwin *et al*., 2003).

Solanum cheesmaniae and *S. galapagense*, the two endemic tomatoes, were separated from each other both morphologically with leaf and fruit characters, fruit characters and allozymatically (having shared and private fixed alleles, Table 3.3). These two endemic species differ from the introduced species in several characters including fruit colour, sepal angle and a suite of flower characters. Fruit colour, however, is the most useful morphological character - the endemic taxa have orange fruit while those of the introduced taxa are red (Tables 3.20. 1-17). *Solanum pimpinellifolium* had a private allele making this species allozymatically unique and *S. lycopersicum* had a unique combination of alleles making it a fourth discrete species, with morphology and allozyme evidence being congruent.

An exciting scientific discovery was that all species were found to form hybrids in the wild in the Galápagos Islands. There were two different categories of hybrids identified within the islands: 1) hybrids that easily can be identified in the field as being morphologically intermediate between two taxa and 2) those that were found to be introgressed/hybridized using genetic markers, but did not show intermediate morphologically.

Solanum cheesmaniae and *S. galapagense*, the two endemic taxa, were found to hybridise on Islas Isabela and Fernandina, where they were found in sympatry. Hybridization is common amongst plants, it is estimated that 25% of plant species are capable of hybridization (Mallet, 2005). Viable and fertile offspring are often the result of these interspecific hybridization events (Lawton-Rauh *et al.,* 2007).

There are many examples of hybridization between endemic species on islands, for example, species in the genus *Scalesia* (Asteraceae) in the Galápagos Islands are known to hybridise when species' ranges overlap, e.g. in *Scalesia divisa* and *S. incisa* on Isla San Cristobál (Nielsen *et al*., 2003). The endemic silversword alliance from the Hawaii Islands have also been widely studied and known to hybridize (Lawton-Rauh, 2007). In some species and circumstances hybrids are found to be more successful than either parent. The Darwin's finches (here as *Geospiza* spp.) on Isla Daphne Major in the Galápagos have been studied by Peter and Rosemary Grant since 1976 (Grant and Grant, 1996). Here they observed hybridization between several species over many years and of particular interest was the low levels of hybridization between *G. fortis* and *G. scandens*. These two finch species were found to produce F1 hybrids which, under 'normal' circumstances, were not observed to successfully reproduce. However the severe El Niño event in 1983 revealed that the hybrids were as successful as or even more successful in reproducing than their pure relatives. The hybrids' intermediate beak size allowed these finches to take advantage of the smaller seeds that became abundant due to the high levels of rainfall caused by the El Niño (Grant and Grant, 1996).

In my study, the most extensive hybrid swarm/zone found during this study was between the endemic *S. cheesmaniae* and the introduced invasive *S.*

pimpinellifolium along the Baltra Road on Isla Santa Cruz, centred around a municipal rubbish dump (Figure 3.11) in this localitiy, three entities, *S. cheesmaniae, S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* hybrids, were found in sympatry. There is a high probability that the rubbish dump could be the site of introduction of *S. pimpinellifolium* to this area or at least act as a stepping stone from Los Gemelos at 600m where *S. pimpinellifolium* was recorded and collected in 1991 (TGRC accession LA3123). Furthermore, this area might also well be the only area where the two parental taxa can both grow together, due to climatic and ecological constraints. *Solanum cheesmaniae* was found to the north of this site and at lower altitudes, in the more arid areas, while *S. pimpinellifolium* was confined to the moister areas south of the Basura dump. The hybrids are found at three other sites both to the north and to the south, thus appear to spread and this is clearly illustrated, and demonstrated, in Figure 3.11. The threat of deleterious genetic effects to populations of *S. cheesmaniae* is of great concern.

Hybridization between endemic and introduced taxa is also common in island groups. In the Canary Islands, several examples of such hybridization have been studied: *Arbutus canariensis*, *Senecio teneriffae* and *Phoenix canariensis* were all found to hybridise with congeneric introduced taxa (Francisco-Ortega *et al*., 2000). It has been suggested that the main threat to *Phoenix canariensis* in the wild is hybridization with the introduced date palm (*P. dactylifera*) (Morici, 1998).

Hybridization can cause introgression and the loss of the genetic integrity of the rarer and endemic species. In addition to this, individuals of the invasive species *S. pimpinellifolium* may inherit potentially beneficial characters from the endemic *S. cheesmaniae* and this might allow the introduced species to become even more invasive by expanding its range ecologically, and ultimately spreading to other islands. Hybridization is often found to be a stimulus for the development of invasiveness (Largiadèr, 2007). In addition to this it is estimated that 25% of invasive hybrids are due to hybridization events between a native and an introduced plant. If invasiveness does evolve this can sometimes take place rapidly (Ellstrand and Schierenbeck, 2006). A long term study of hybridization within *Helianthus* spp. (sunflowers) gave an example of

invasiveness after intertaxon hybridization between *Helianthus annuus* x *H. debilis* spp. *cucumerifolius* with the result a new taxon, *H. annuus* spp. *texanus*, that is invasive (Ellstrand and Schierenbeck, 2006).

The Galápagos tomatoes are not only important as they are rare endemics from this iconic island group but also because they are close relative of an extremely important global crop plant. These plants could hold useful characters for future generations of plant breeders. Seed bank accessions may not hold the key to conservation of crop plants due to the problems associated with long-term storage of seeds. More importantly, the preservation of crop relatives in the wild where they can continue evolving in a natural habitat with natural pests and symbionts will ultimately be important for crop improvement in the future. Hybridization between crop plants and their wild relatives is also widely studied, and has been the subject of book length treatments (e.g. Ellstrand, 2003). There are several problems that can arise from crop plants hybridizing with wild relatives. For example they can cause problems for the crop yield as was the case with the 'bolting beet', a weedy hybrid formed within the crop field between the sea beet (*Beta vulgaris* subsp. *maritima*) and the cultivated sugar beet (*B.vulgaris* subsp. *vulgaris*), causing significant problems and reduction of profits (Ellstrand, 2003). Conversely there are many examples of where the genes from crop plants have also been found to escape into wild plant populations, for example in the sunflowers, *Helianthus* spp. (Ellstrand, 2003).

It is obvious from a comparison of historical and recent records that the current distribution of *S. cheesmaniae* on Isla Santa Cruz is much reduced in extent. Collections on most islands were made by and on behalf of the TGRC from 1950s onwards. Many of these early tomato localities for example from Academy Bay/Puerto Ayora/Tortuga Bay and surrounding areas towards the central part of the island were not found by me during my extensive fieldwork in 2000 and 2002 nor are they reported by Nuez *et al.* (2004). Indeed, during my field collection excursions towards the south of Santa Cruz only one individual plant of *S. cheesmaniae* was collected (SCD accession 777 from near the CDRS Entomology building which was one of the 'jointless' types, that have been used for crop improvement in the past). The loss of *S. cheesmaniae* in Puerto Ayora (Isla Santa Cruz) is probably due to the extensive urbanization of

this area and maybe also due to goat predation (Nuez *et al*., 2004, and pers. obs.). However there are some areas that have not been built on and are inaccessible to goats around the barrancos (rocky outcrops), but these did not harbour tomatoes in 2000 or 2002. Furthermore, individuals of *S. pimpinellifolium* are ubiquitous in Puerto Ayora. This indicates a real threat through hybridization to any populations of *S. cheesmaniae* that may still be left in this area. Further thorough searches for *S. cheesmaniae* should take place during different seasons and research into the genetic and morphological status of this species should be ongoing.

Several other islands also have introduced and endemic tomatoes growing on or near them. Islas Isabela and San Cristóbal both support populations of endemic tomatoes as well as introduced tomatoes, raising the threat of hybridization between the endemic and the introduced species on these islands too. Although endemic tomatoes have not been collected from Isla Floreana, populations of *S. galapagense* do occur on two neighbouring islets - Corona del Diablo (300m from the coast of Isla Floreana) and Gardner (1km from the coast of Floreana). The presence of *S. lycopersicum* on Isla Floreana could also potentially pose a threat to the Corona del Diablo populations.

The problem with seed banks

Novel allelic variations were found within the seed bank accessions that were not found in the wild populations in the Galápagos Islands. This raises the possibility of some introgression within the seed bank collections during rejuvenation trails. I would have strong concerns in relation to the suggestion made by Nuez *et al*. (2004) that reintroductions of seed bank accessions to the Galápagos Islands could restore tomato populations, if they became extinct in the wild. Hybridization in seed banks also raises general questions of the long-term viability of storing seeds in this way. It furthermore provides strong arguments for the conservation of wild crop relatives in natural habitats. This is probably the only safeguard for organisms and their use by future generations of biologists, natural historians and plant breeders. Furthermore, serious scientific studies can be at risk from misidentification or genetic erosion in

accessions, as many seed bank accessions are being used all over the world for expensive and extensive genetic studies and plant breeding programmes.

Dispersal

During the course of this field work and analysis of the collection localities of herbarium specimens, many populations of Galápagos tomatoes grow in areas where giant tortoises do not occur at the moment and almost certainly never did, for example islets Bartolomé and Sombrero Chino off the coast of Isla Santiago and Corona del Diablo off the coast of Isla Floreana. Therefore, tomato dispersal and the breaking of seed dormancy cannot be exclusively undertaken by giant tortoises, as already suggested by Rick and Bowman (1961). Significant percentages of seeds (up to over 50%, n= 15, after two months) germinated without the any seed treatment (pers. obs. and Rick and Bowman, 1961, but they report a lower percentage). Anne Schultz (pers. comm. 2000) undertook research into seed species diversity in tortoise excrement from El Chato Tortoise Reserve in 2000. She reported that she did not find any tomato seeds in these excrements. Interestingly, there was a large population of *S. pimpinellifolium* and it may well be that Galápagos tortoises do not eat red-fruited tomatoes, despite them being more palatable for humans. This however needs further investigation

The two endemic species of Galápagos tomatoes have smaller seeds (1.5-2.2mm long) than either *S. lycopersicum* (3.5mm long) or *S. pimpinellifolium* (2-3mm long) (Darwin *et al*., 2003). Nakazato *et al*. (2008) writes that differences in seed size can indicate different dispersal mechanisms, for example small seed size might indicate dispersal by birds. I have observed mockingbirds carrying (but not eating) *S. pimpinellifolium* fruit on Isla Santa Cruz (as have Nuez *et al*., 2004). In 2002 I fed some captive-bred Darwin's finches tomato seeds treated with red food dye. The seed paste and food dye reappeared later in the excrement having been ground up in their gizzards, thus I concluded that finches couldn't be responsible for tomato seed dispersal via their gut.

Rick and Bowman (1961) suggest that goats may be responsible for tomato seed dispersal. I observed tomato seedlings (not identified to species level)

growing out of either donkey or goat excrement at ‘El Mango’ a gravel pit near Villamil on Isabela (00˚52’94”S 91˚00’70”W).

The future of the Galápagos tomatoes – conservation issues

Allendorf *et al*. (2001) provided a framework to categorize different types of hybridization. They outline six types of hybridization *Types 1-3* they describe as natural hybridization and *Types 4-6* are anthropogenic hybridization. This is where humans have been responsible either directly or indirectly by bringing the previously naturally separated plants or other organisms together. The *S. cheesmaniae* x *S. pimpinellifolium* hybrid populations along the Baltra Road on Isla Santa Cruz could be described as *Type 5* (*sensu* Allendorf *et al*., 2001). This category describes taxa where the F1 generation is fertile and hybridization and introgression is widespread, however some populations remain pure. The Allendorf *et al*. (2001) conservation recommendations for *Type 5* were to maintain and expand any existing pure populations and they suggested that the hybrid populations are of little conservation value. The question of whether hybrid populations are of conservation value is an interesting one, where few pure populations remain then it is preferable to maintain the hybrid populations as this conserves at least some of the parental genomes. Given the fact that I class *S. pimpinellifolium* as a highly invasive species (see Chapter 1) my recommendations for the conservation of *S. cheesmaniae* from Santa Cruz would be that the hybrids and *S. pimpinellifolium* should be eliminated and that pure lines of the different populations from nearby natural sites (e.g. North Coast and ‘jointless’ from Puerto Ayora) should be grown in *in-situ* conservation projects.

Nuez *et al*. (2004) noted that the largest populations of Galápagos tomatoes were found in areas where there are no feral goats and donkeys (for example Bartolomé and Sombrero Chino); they observed that on Baltra and Santiago (with ample feral grazers) tomato plants were confined to goat inaccessible crevices. I made the same observations during my extensive fieldwork, but there were exceptions of a few areas on Isabela near Villamil on Isla Isabela and on Isla Santa Cruz, where I found flourishing populations of tomatoes despite the presence of goats (at the time). It may be that in the presence of

other more palatable food sources persuaded the goats to avoid the bitter-tasting tomato plants.

During their fieldwork in the Galápagos Islands, Nuez *et al*. (2004) only found two individual plants of *S. galapagense* on the small islet of Corona del Diablo (200 m off Isla Floreana). Isla Floreana is currently undergoing considerable conservation efforts to rid the island of feral animals. *Solanum galapagense* almost certainly once occurred on Floreana (according to a former resident of Floreana it still does grow there; F. Cruz, pers. comm. 2009) and this population on Corona del Diablo may well represent the remnant of a much larger former population on Floreana. These plants on Corona del Diablo need to be safe-guarded and if necessary, seeds collected for an *in situ* conservation programme on Isla Floreana itself. If/when, at a later stage, the historical occurrence of tomatoes from Floreana could be confirmed; populations might be restored onto that island. Islote Gardner near Isla Floreana also had a population of *S. galapagense* (Rick 1971) however this islet is about 1km away from Isla Floreana. There is a similar situation on Isla Española where *S. galapagense* is only found on a small islet to the north, also called Islote Gardner.

Further field investigations should be undertaken in the Puerto Ayora area to see if, in particular, further plants of the 'jointless' type can be can be located. Seeds might be collected and grown up in the local native plant nursery at the Charles Darwin Research Station (CDRS). Initially plants could be grown round the Parque Nacional Galápagos (PNG) and CDRS offices while *S. pimpinellifolium* are removed from this area. There is an existing native plant programme in Puerto Ayora (Isla Santa Cruz) that aims to encourage local householders to grow suitable native plants in their gardens. *Solanum cheesmaniae* would be a good candidate for this once *S. pimpinellifolium* is removed from the area as it is an attractive plant and native to this exact area.

Nuez *et al*. (2004) suggested that some *ex situ* conservation might be advisable to preserve existing populations. They suggest that TGRC seed accessions might be used to replace populations currently missing the wild. I would suggest great caution and advice further tests on the seed bank accessions to

check for the genetic integrity prior to using any seed bank accessions for any form of restoration project in the wild. Using seeds from existing wild stock is far more preferable. However Francisco-Ortega *et al*. (2000) regard transporting individual plants to areas where the species no longer exists should be done only as a last resort, so further research should be undertaken before this action is taken.

Future work

I have approached CDRS with a request to undertake an experiment with captive-bred mockingbirds to assess if they will eat tomatoes and to assess seed germination. Mockingbirds might be a good candidate for local tomato dispersal and dispersal between the islands.

Comparative genetic studies of invasive species from their native range and the introduced range will yield more information on the importance of genetic variation in plants invasions (DeWalt and Hamrick, 2004). There already is a lot of research including genetic studies on wild populations of *S. pimpinellifolium* (for example Caicedo and Schaal, 2004) due to its economic importance as a close relative of *S. lycopersicum*. A comparison between the invasive and weedy populations of *S. pimpinellifolium* within the Galápagos Islands in conjunction with these existing studies might make a very interesting research project and might well serve as a model study for plant invasions. Charles Darwin (1859) wrote that “species which are most numerous in individuals will have the best chance of producing within any given period favourable variations”. This indicates that Darwin understood even then that the more individuals belonging to an invasive species, the greater the potential is for it to become more widespread and thus more problematic.

The western islands of Islas Fernandina and Isabela lie in the direction of the ocean currents, and although Kurth *et al*. (1986) found that seeds of Galápagos tomatoes did not germinate well after being immersed in seawater it might be worth revisiting this research. The seeds used in Kurth *et al*. (1986) were fully imbibed prior to immersion in salt water. Charles Darwin (1857) tested tomato

seed germinability after seawater treatment. Although Darwin's results showed low levels of germination he cited the work of a Mr. Berkeley who had apparently found that tomato seeds germinated after 30 days in seawater. Future research to test for the possibility of the endemic tomato dispersal by seawater could be undertaken on un-imbibed seeds.

Isla Isabela is over 120 km long (three times longer than any other island in the Galápagos). Larger islands tend to have more species and genetic diversity. Even if there are volcanic activities in one of the four active volcanoes on this island the plants can re-colonise rapidly with the help of land birds and reptiles. Tomatoes are often found as early colonisers. I observed on the northwest side of Fernandina that tomatoes were one of the few plant species on recent lava flows. Isla Isabela is less than 5km away from the northeast corner of Fernandina. A detailed genetic study of these tomato populations would be an interesting study of dispersal and re-colonisation events after volcanic eruptions.

Solanum pimpinellifolium

Solanum pimpinellifolium is described as an invasive species *sensu* Rejmanek (Darwin *et al*., 2003) and while it may be advantageous for an invasive plant to be genetically diverse to be able to adapt to a different range of environmental conditions to those experienced within its native range. A 'multipurpose genotype' allows the species to grow in many different habitats might also be advantageous with respect to characteristics such as phenotypic plasticity (DeWalt and Hamrick, 2004). Certainly during the course of this study *S. pimpinellifolium* was found in an extremely wide range of habitats within Isla Santa Cruz, from the arid zone, with high light levels, to near sea level at Puerto Ayora and to the humid zone with lower light levels at the fringes of cloud forest at Los Gemelos near the summit of this island. I collected it as a roadside ruderal, it proliferated at the rubbish dump located just off the Baltra Road where it overflows into the native vegetation which surround the rubbish dump, and in the gravel mine Mina Roja. It is found in natural habitats, for example at El Chato where there seems to be little disturbance apart from a population of giant tortoises and a tourist trail.

Conclusion

Allozyme electrophoresis supported the morphological species delimitation of *S. cheesmaniae*, *S. galapagense*, *S. pimpinellifolium* and *S. lycopersicum* as published by Darwin *et al*. (2003); fixed differences or unique combinations were elucidated using these markers for all the tomato taxa in the Galápagos Islands. Within all groups of tomatoes in the Galápagos Islands there are very low levels of diversity, low levels of out-breeding and a strong departure from Hardy-Weinberg equilibrium.

Allozyme electrophoresis also corroborated the morphological intermediacy of hybrid populations. Of particular interest is the hybrid zone between the endemic *S. cheesmaniae* and the invasive *S. pimpinellifolium*, discovered along the Baltra Road in Isla Santa Cruz. In addition to this northern Baltra Road area there are other areas of concern for the potential hybridization between native and introduced tomatoes on Isla Santa Cruz, the most densely populated island in the archipelago. There were indications that a single coastal plant of *S. cheesmaniae* from the northeast of the island had allelic variation derived from *S. pimpinellifolium* and further indications that the *S. cheesmaniae* found on Isla Pinzón may be introgressed with *S. pimpinellifolium*. Although this could be viewed as insignificant this might also be an indication of further populations of hybrids in the surrounding area - as yet uncollected. A thorough search of the area to the east of the Baltra Road towards the northeast coastal populations should be undertaken with urgency.

Solanum pimpinellifolium occurs extensively around Puerto Ayora (Isla Santa Cruz), both as individual plants and in large patches. *Solanum pimpinellifolium* samples were also collected east of the town towards the offices of GNP and CDRS in Puerto Ayora. *Solanum cheesmaniae* populations were once common in the Academy Bay region (Puerto Ayora). Plants from this population possess a morphological character known as ‘jointless’ (e.g. TGRC accession LA0166) (Rick, 1967). During my fieldwork (2000 and 2002) I only found a single plant of *S. cheesmaniae* in 2002 from Puerto Ayora. This plant was located by the

CDRS buildings in Puerto Ayora. This was despite a thorough search of the area extending both east and west of Puerto Ayora. The close proximity of the *S. pimpinellifolium* to this plant of *S. cheesmaniae* (500m) poses a significant threat to this important population. Nuez *et al*. (2004) also did not find any Galápagos tomatoes in this region during their fieldwork in 2000.

The report by Nuez *et al*. (2004) that there are only two individual plants of *S. galapagense* found on Corona del Diablo (off Isla Floreana) is of great concern for the survival of this population. This population may represent the remaining remnant of a larger population on Isla Floreana. (along with Isla Gardner to the east). I have applied to get a permit from Parque Nacional Galápagos in order to make collections of this Corona del Diablo population during my field work as part of the Darwin Now in early 2010. A seed collection from these plants should be made with a view of undertaking *in situ* conservation on Isla Floreana at a later date perhaps this could be incorporated as part of Project Floreana (for details of the project see http://www.galapagos.org).

The presence of hybrids between the introduced and endemic tomatoes on the islands poses a significant threat to the rarer endemic species. This could in time result in introgression and a loss of rarer genotypes. There are numerous examples of hybrids between native taxa and introduced taxa becoming invasive species themselves (Ellstrand and Schierenbeck, 2006). Evidence of hybridization between *S. pimpinellifolium* and *S. cheesmaniae* raises a serious cause for concern for the genetic integrity and future of *S. cheesmaniae* on Isla Santa Cruz. Recent communications with Mark Gardener (CDRS director of Terrestrial Science) have alerted me that there is a large increase in *S. pimpinellifolium* in this Baltra Road area on Santa Cruz and with the help of a British Council Grant under their ‘Darwin Now’ I will be able to study this in early 2010.

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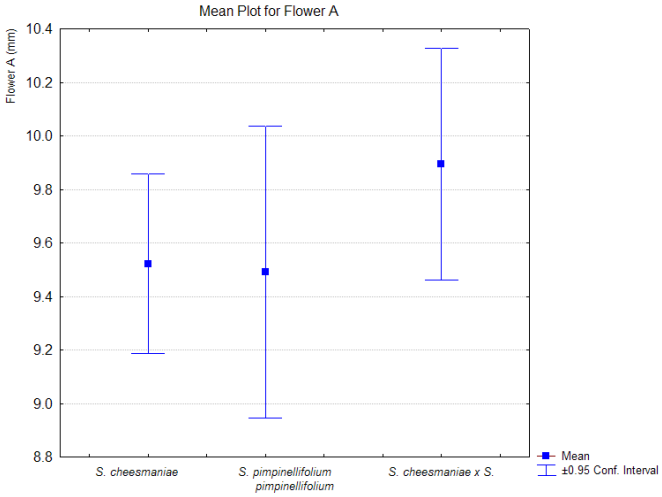
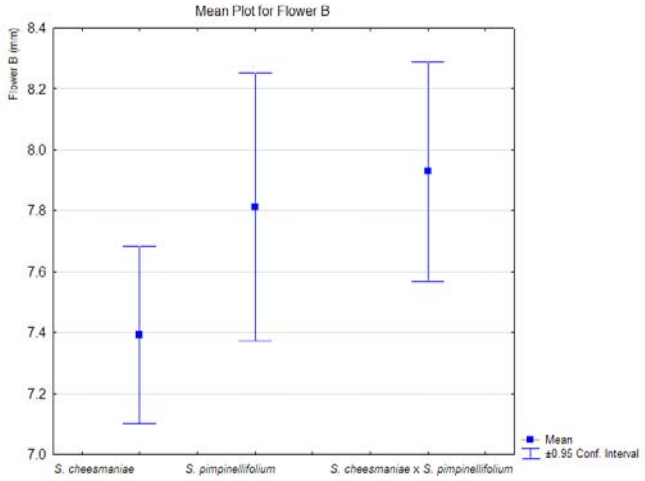
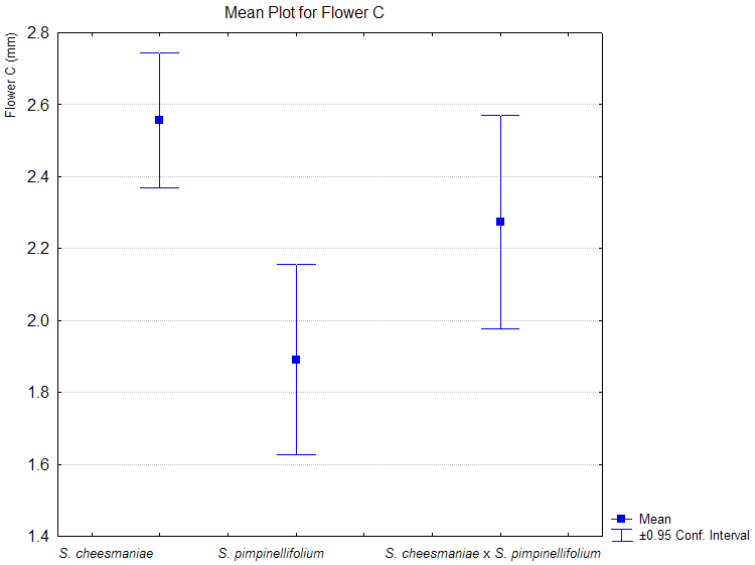
Appendix 1.

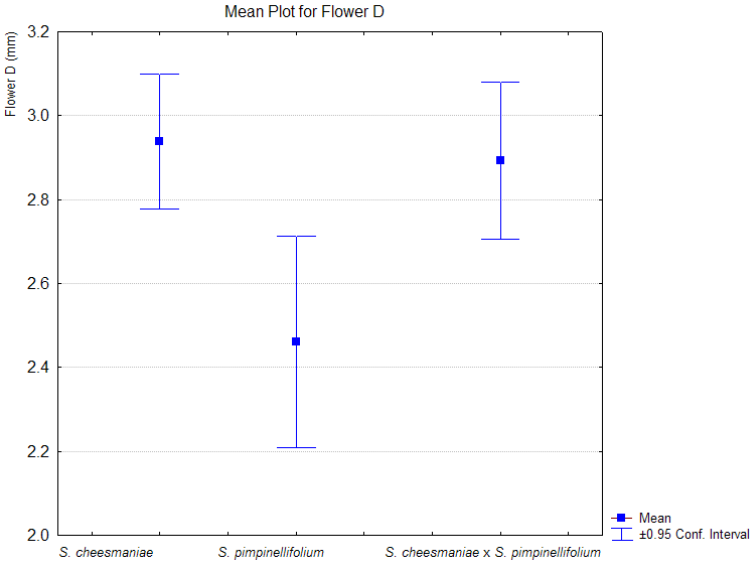
Two different sets of box plots are presented here.

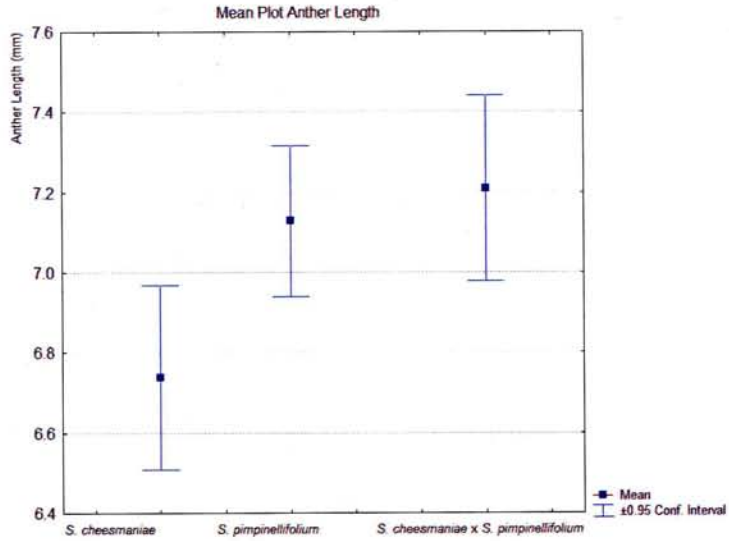
The following 18 figures show the means and 95% Confidence intervals for the morphological characters used to discriminate between *Solanum cheesmaniae*, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* from the Baltra Road hybrid populations, *S. cheesmaniae* from El Lagoon de Manzanilla and *S. pimpinellifolium* from Garapaterra Road, Isla Santa Cruz..

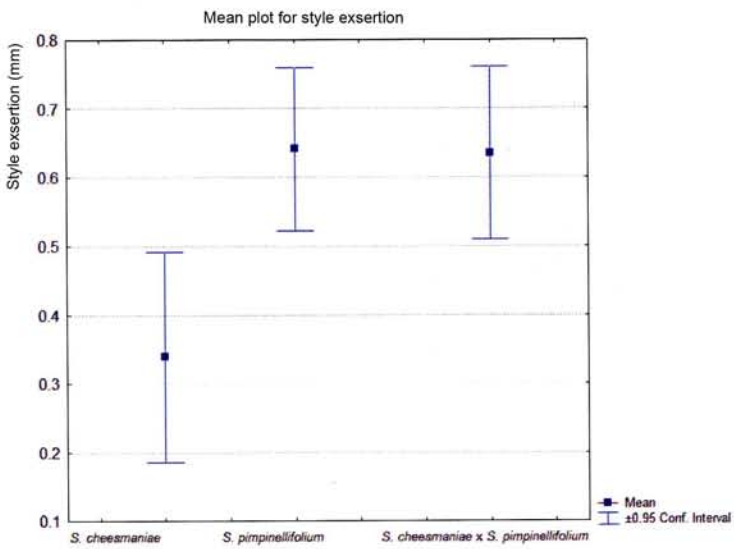


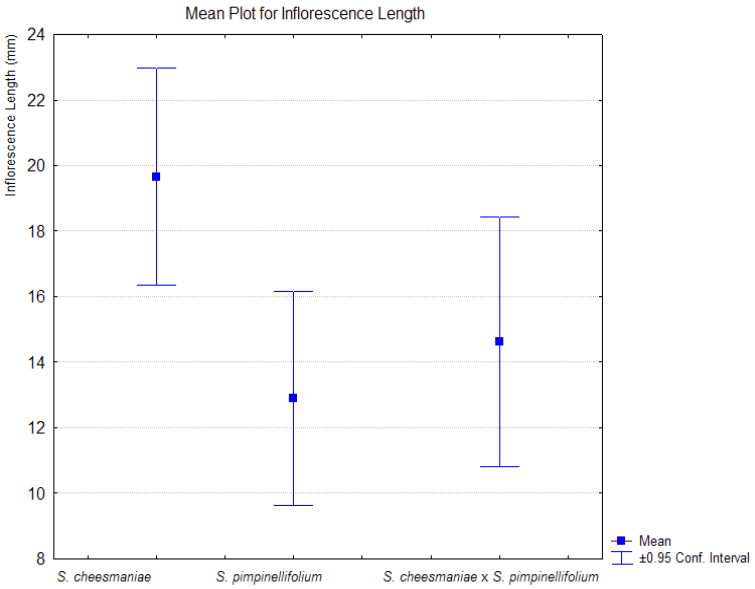


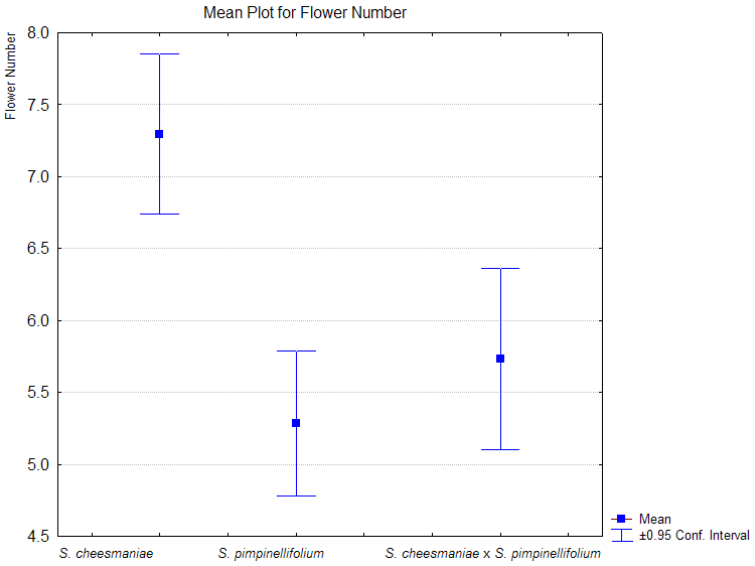

Mean Plot for Flower C
Flower C (mm)
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval

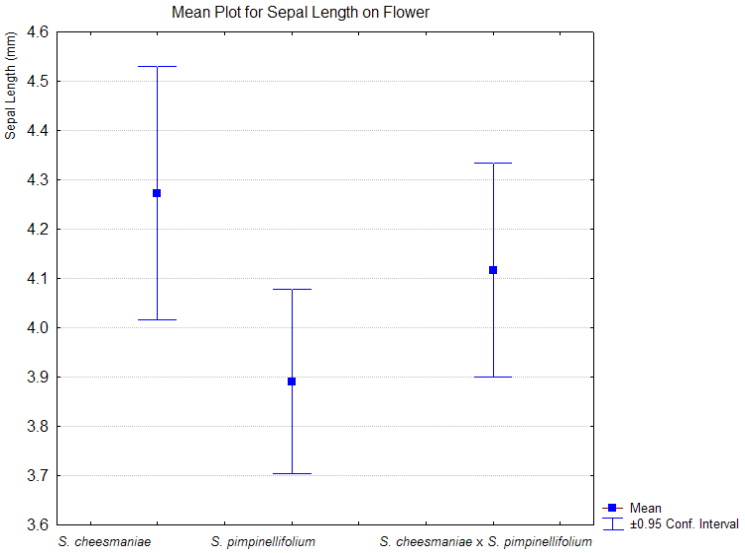

Mean Plot for Flower D
Flower D (mm)
3.2
3.0
2.8
2.6
2.4
2.2
2.0
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval

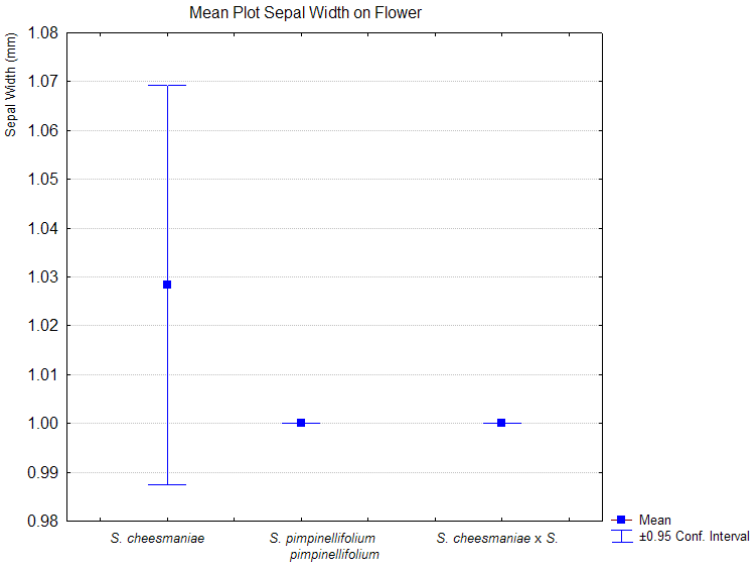

Mean Plot Anther Length
Anther Length (mm)
7.6
7.4
7.2
7.0
6.8
6.6
6.4
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval

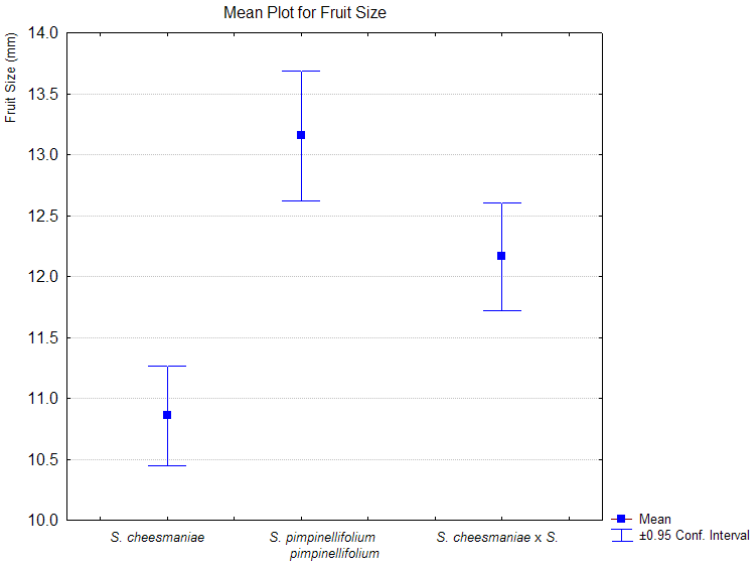

Mean plot for style exsertion
Style exsertion (mm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval

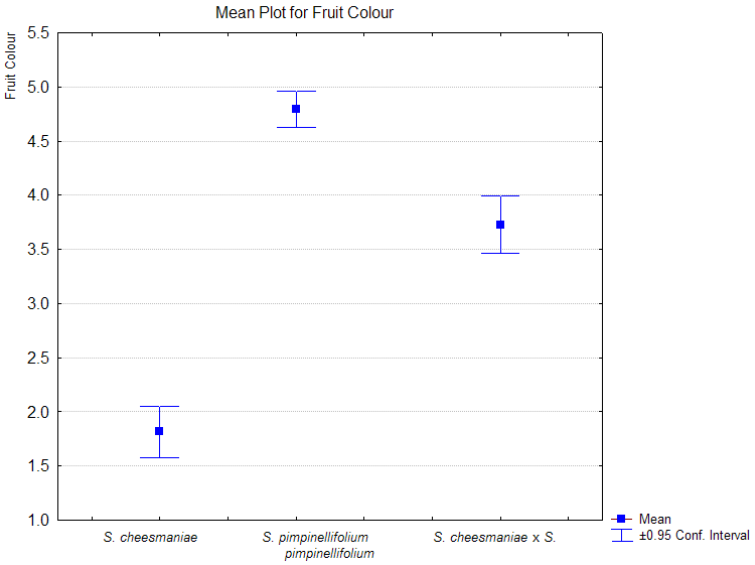

Mean Plot for Inflorescence Length
Inflorescence Length (mm)
24
22
20
18
16
14
12
10
8
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval

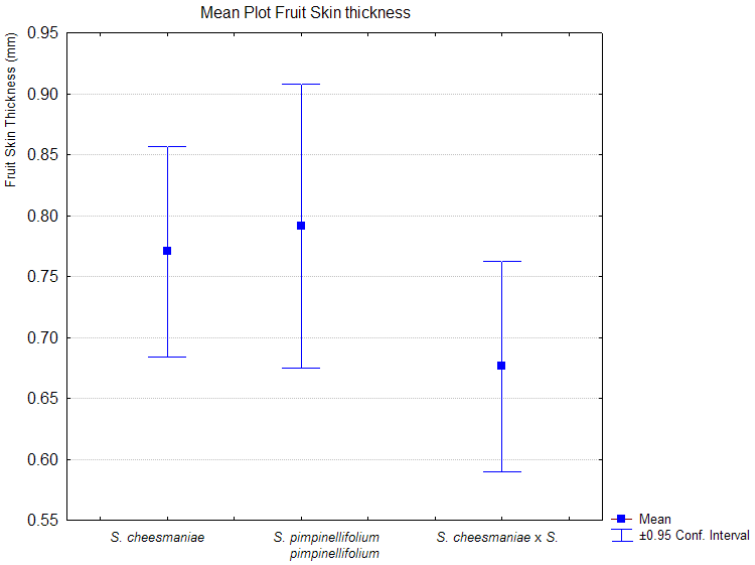

Mean Plot for Flower Number
Flower Number
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval

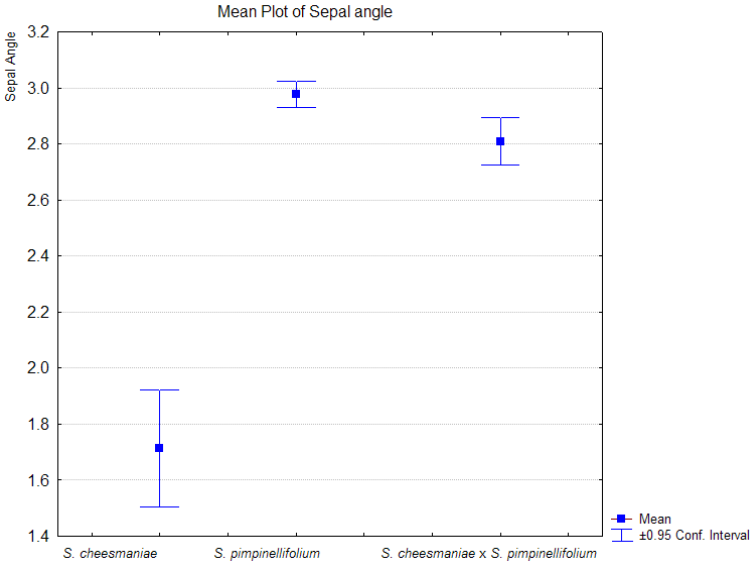

Mean Plot for Sepal Length on Flower
Sepal Length (mm)
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval

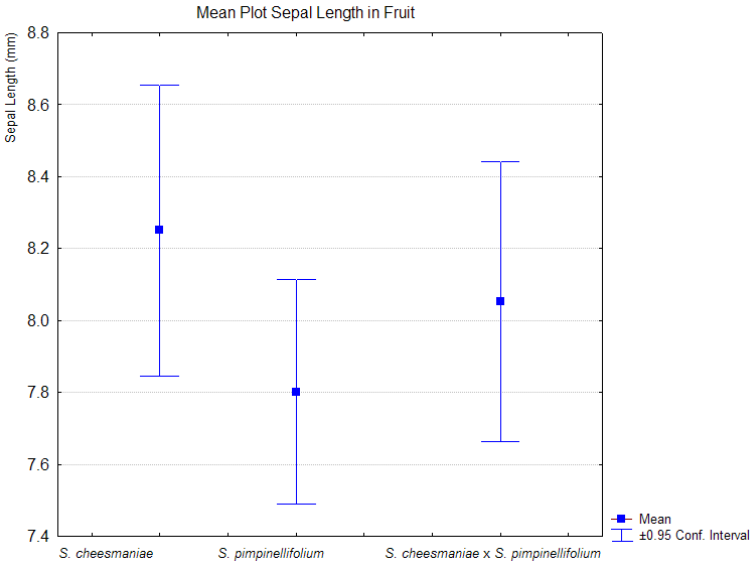

Mean Plot Sepal Width on Flower
Sepal Width (mm)
1.08
1.07
1.06
1.05
1.04
1.03
1.02
1.01
1.00
0.99
0.98
S. cheesmaniae
S. pimpinellifolium
pimpinellifolium
S. cheesmaniae x S.
Mean
±0.95 Conf. Interval

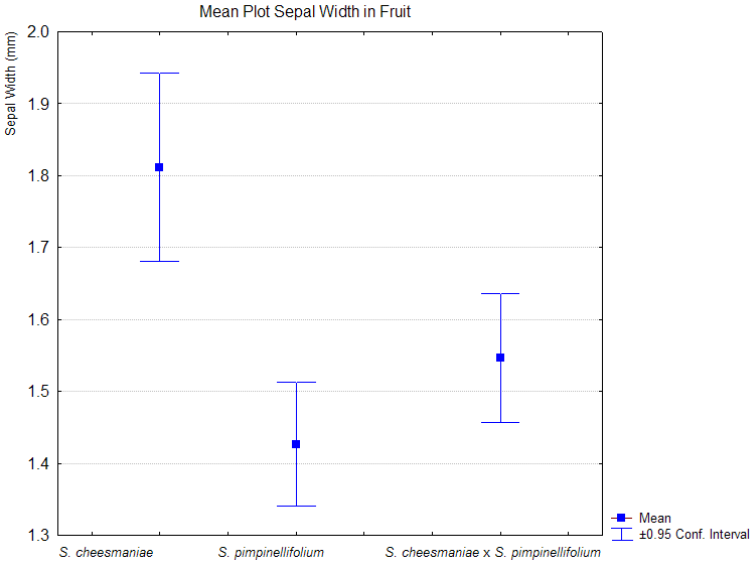

Mean Plot for Fruit Size
Fruit Size (mm)
14.0
13.5
13.0
12.5
12.0
11.5
11.0
10.5
10.0
S. cheesmaniae
S. pimpinellifolium
pimpinellifolium
S. cheesmaniae x S.
Mean
±0.95 Conf. Interval

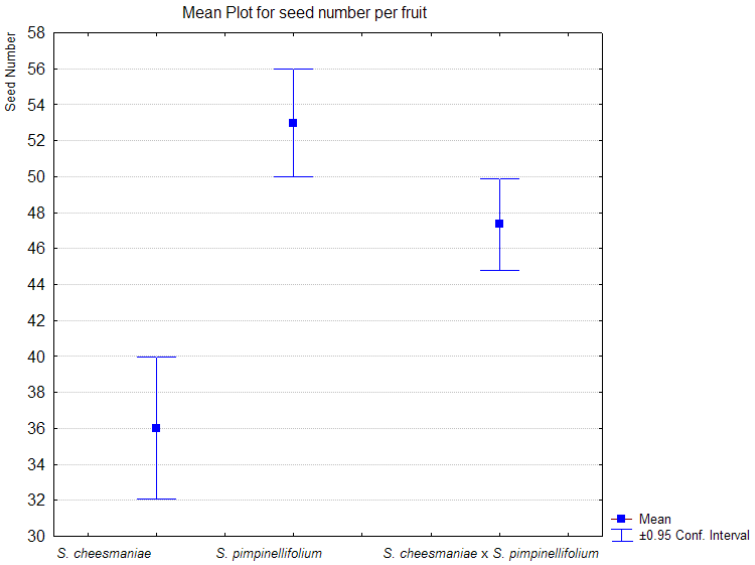

Mean Plot for Fruit Colour
Fruit Colour
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
S. cheesmaniae
S. pimpinellifolium
pimpinellifolium
S. cheesmaniae x S.
Mean
±0.95 Conf. Interval

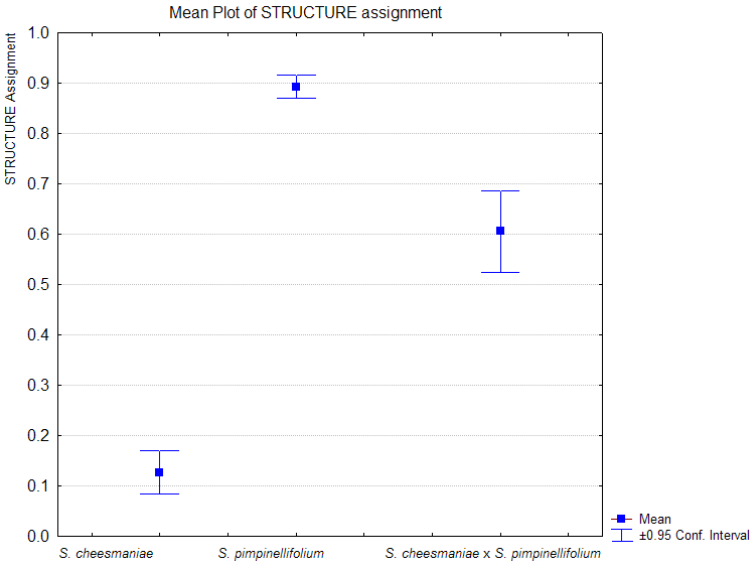

Mean Plot Fruit Skin thickness
Fruit Skin Thickness (mm)
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
S. cheesmaniae
S. pimpinellifolium pimpinellifolium
S. cheesmaniae x S.
Mean
±0.95 Conf. Interval


Mean Plot of Sepal angle
Sepal Angle
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval


Mean Plot Sepal Length in Fruit
Sepal Length (mm)
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval

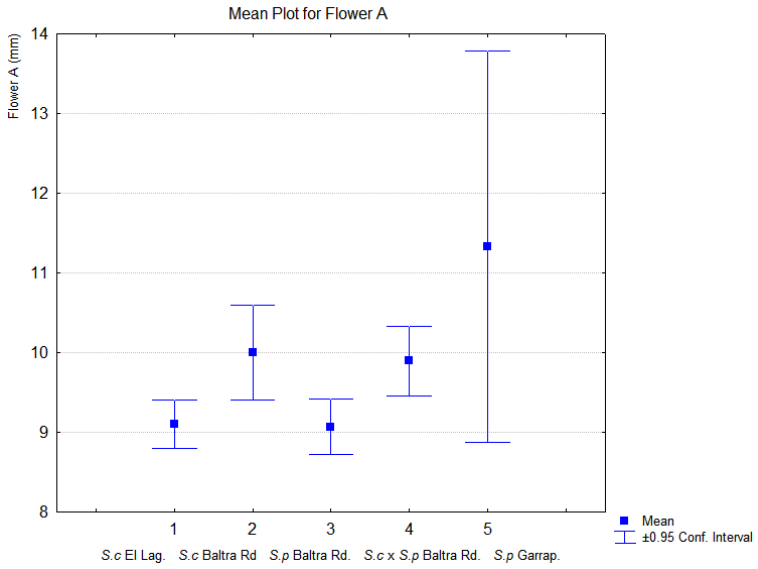

Mean Plot Sepal Width in Fruit
Sepal Width (mm)
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval

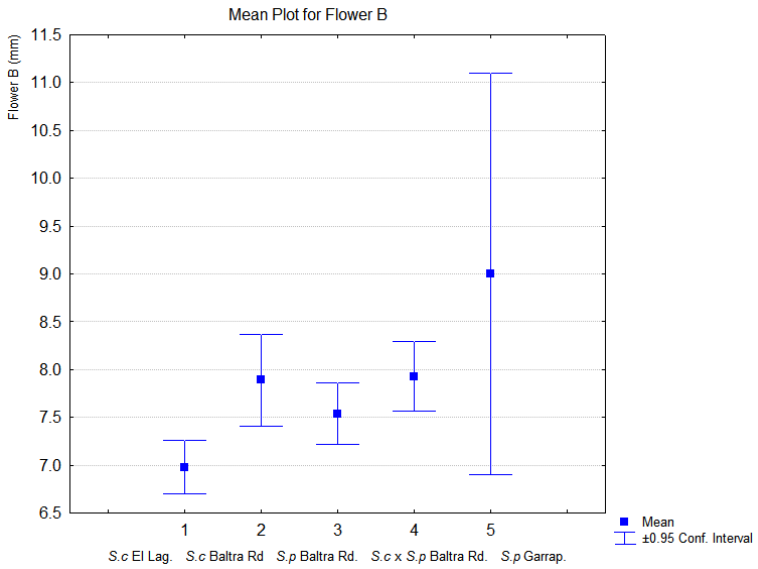

Mean Plot for seed number per fruit
Seed Number
58
56
54
52
50
48
46
44
42
40
38
36
34
32
30
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval

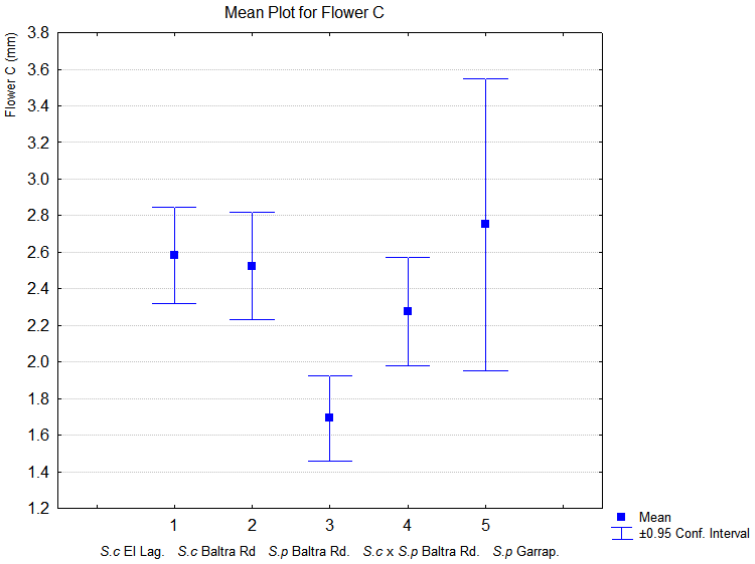

Mean Plot of STRUCTURE assignment
STRUCTURE Assignment
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
S. cheesmaniae
S. pimpinellifolium
S. cheesmaniae x S. pimpinellifolium
Mean
±0.95 Conf. Interval

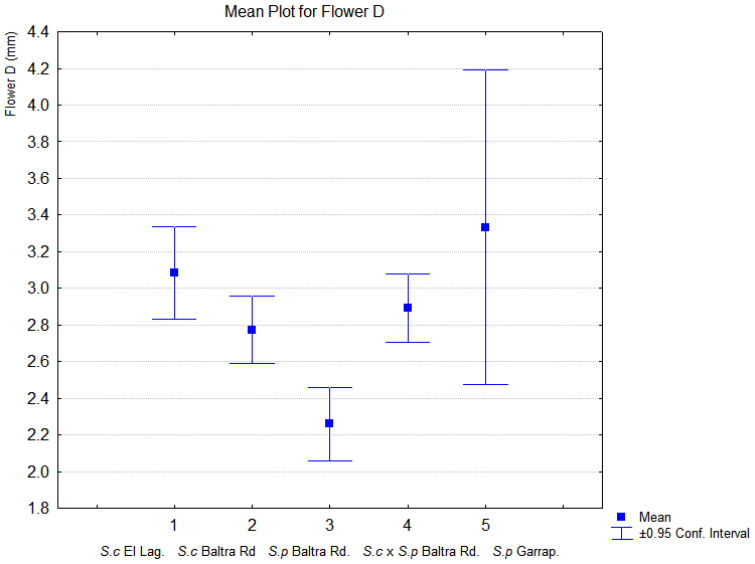
The following 18 figures show the means and 95% Confidence intervals for the morphological characters used to discriminate between *Solanum cheesmaniae*, *S. pimpinellifolium* and *S. cheesmaniae* x *S. pimpinellifolium* from the Baltra Road hybrid populations, *S. cheesmaniae* from El Lagoon de Manzanilla and *S. pimpinellifolium* from Garapaterra Road, Isla Santa Cruz.

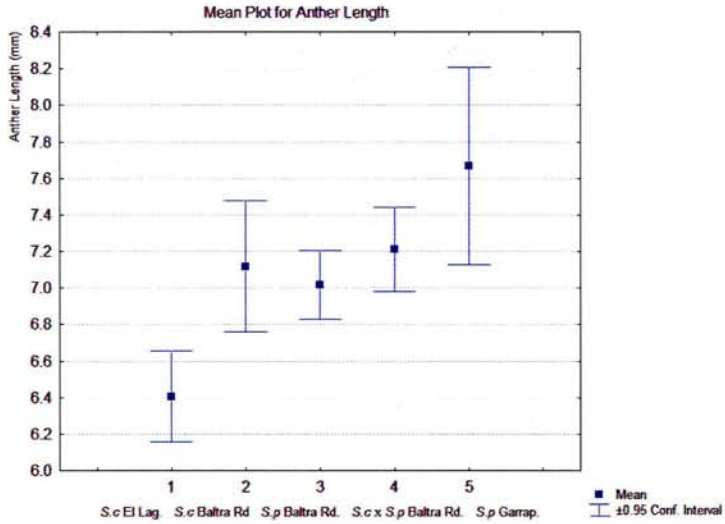
Legend: *S.c* El Lag = *S. cheesmaniae* from El Lagoon de Manzanilla, *S.c* Baltra Rd. = *S. cheesmaniae* from Baltra Road, *S.p* Baltra Road = *S. pimpinellifolium* from Baltra Road; *S.c* x *S.p* Baltra Road = *S. cheesmaniae* x *S. pimpinellifolium* from Baltra Road; *S.p* Garap. = *S. pimpinellifolium* from Garapaterra Road.

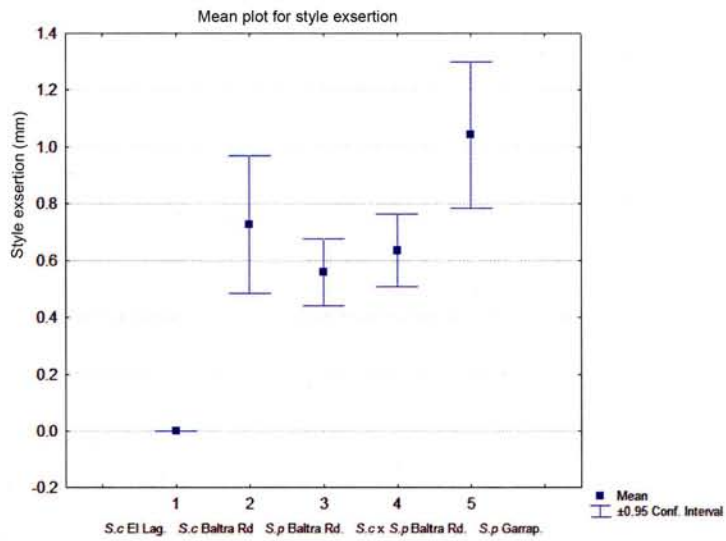


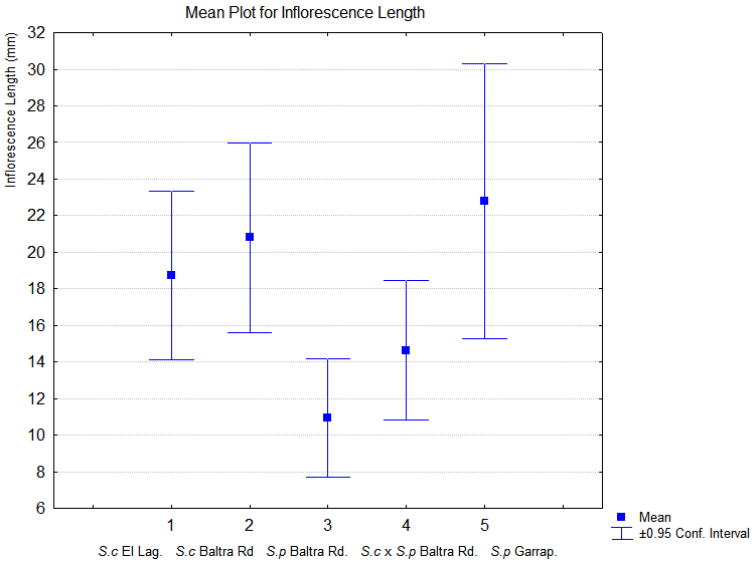


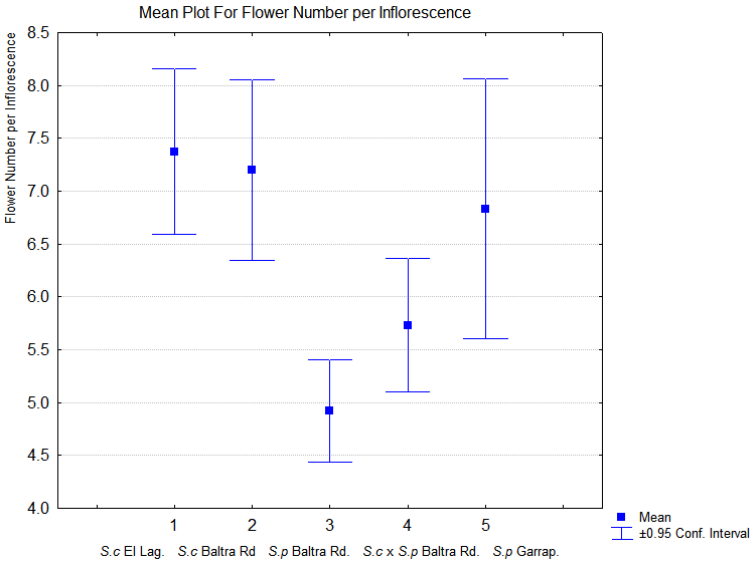

Mean Plot for Flower C
Flower C (mm)
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval

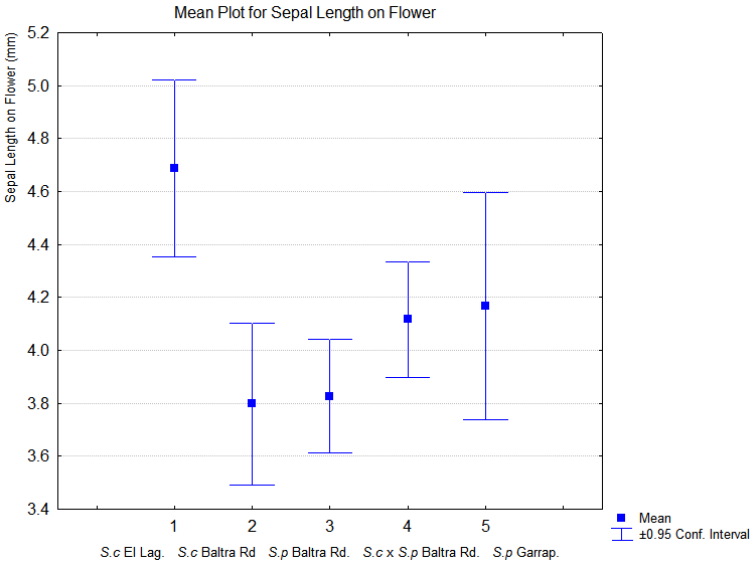

Mean Plot for Flower D
Flower D (mm)
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval

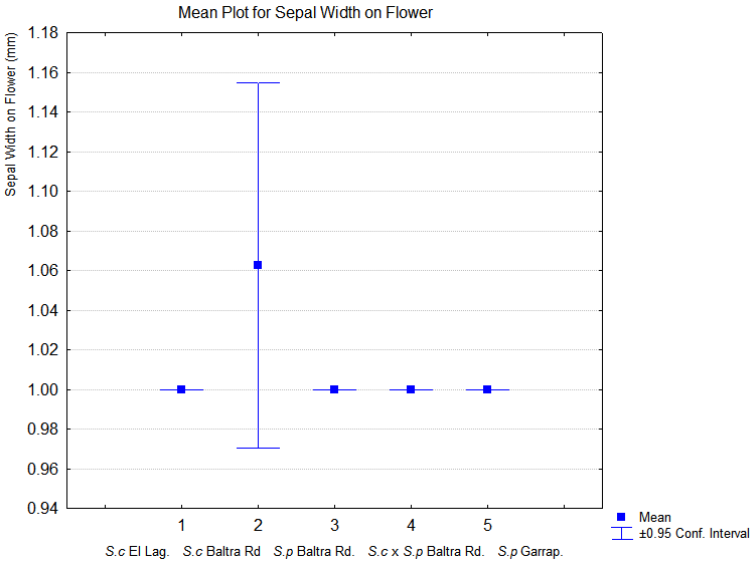

Mean Plot for Anther Length
Anther Length (mm)
6.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval

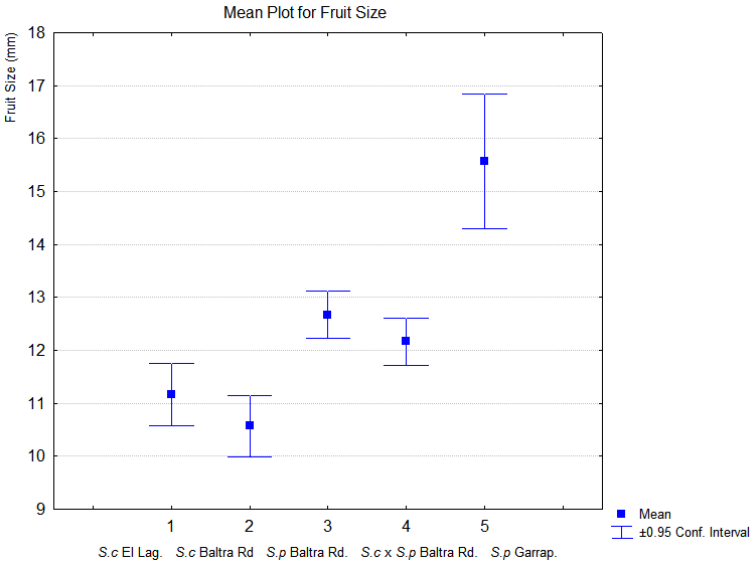

Mean plot for style exsertion
Style exsertion (mm)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval

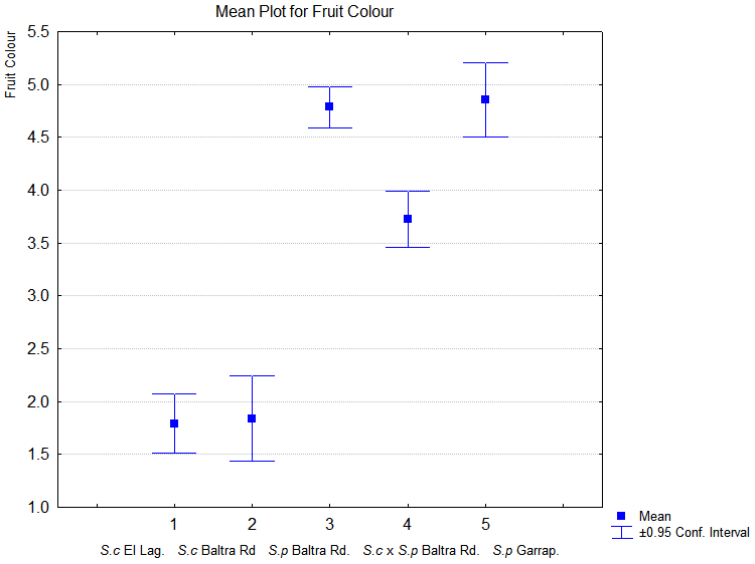

Mean Plot for Inflorescence Length
Inflorescence Length (mm)
32
30
28
26
24
22
20
18
16
14
12
10
8
6
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval

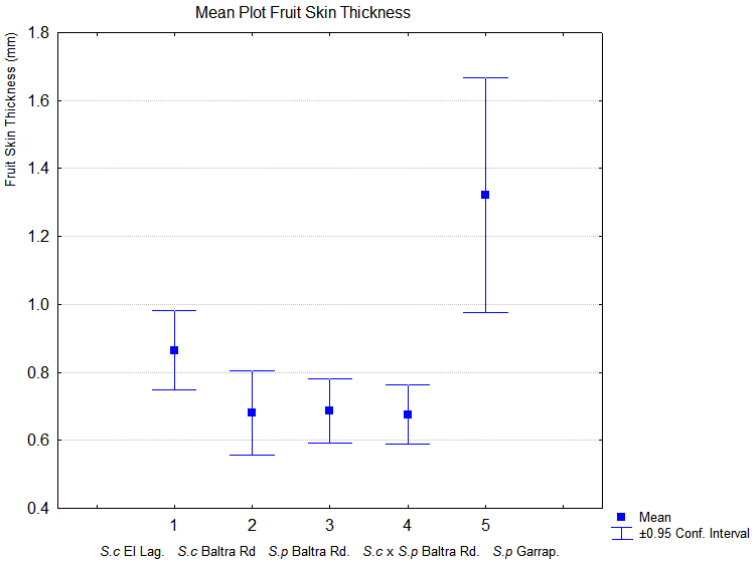

Mean Plot For Flower Number per Inflorescence
Flower Number per Inflorescence
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval


Mean Plot for Sepal Length on Flower
Sepal Length on Flower (mm)
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval

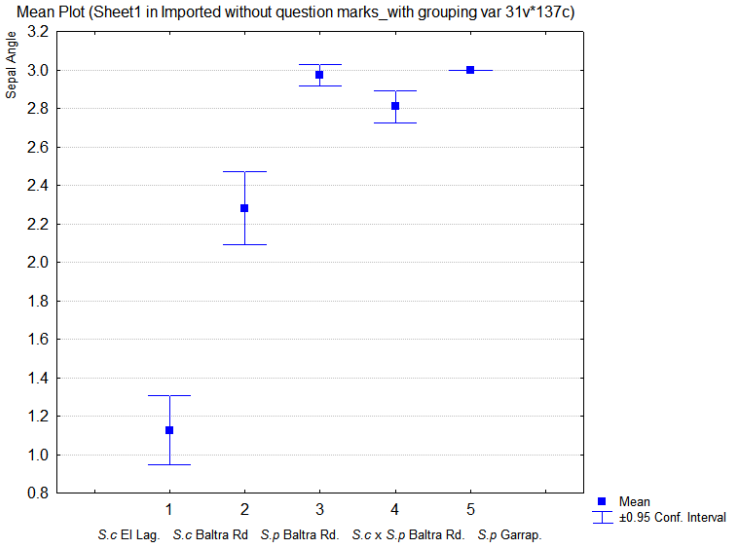

Mean Plot for Sepal Width on Flower
Sepal Width on Flower (mm)
1.18
1.16
1.14
1.12
1.10
1.08
1.06
1.04
1.02
1.00
0.98
0.96
0.94
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval

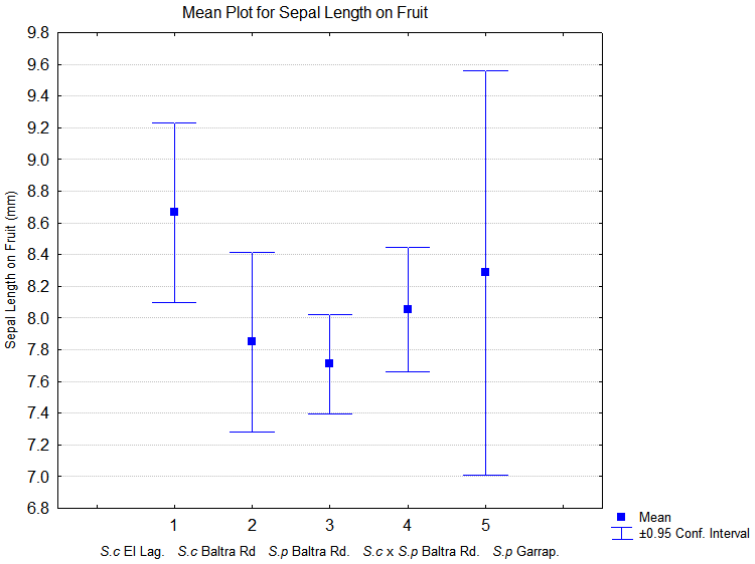

Mean Plot for Fruit Size
Fruit Size (mm)
18
17
16
15
14
13
12
11
10
9
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval

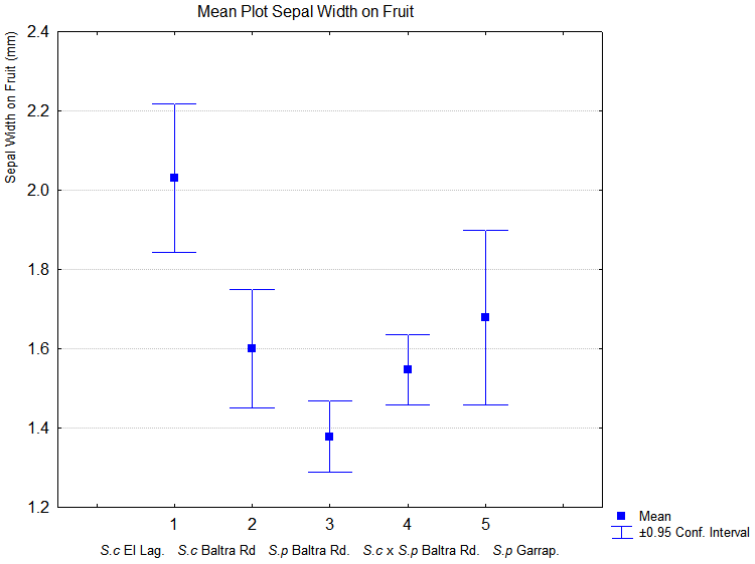

Mean Plot for Fruit Colour
Fruit Colour
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval

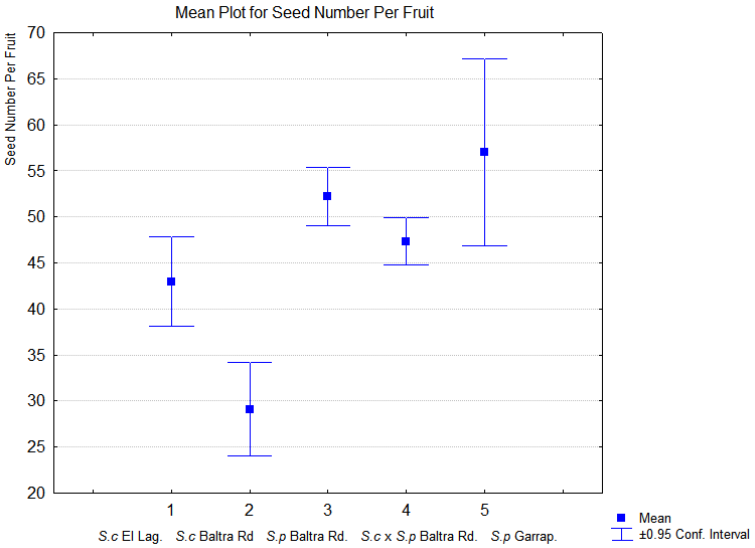


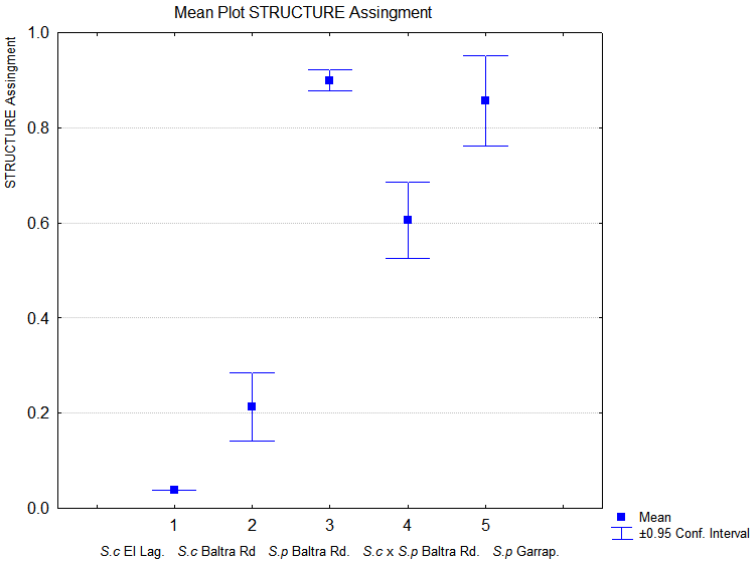
Mean plot Sepal angle




Mean Plot for Sepal Length on Fruit
Sepal Length on Fruit (mm)
9.8
9.6
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval


Mean Plot Sepal Width on Fruit
Sepal Width on Fruit (mm)
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval


Mean Plot for Seed Number Per Fruit
Seed Number Per Fruit
70
65
60
55
50
45
40
35
30
25
20
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval


Mean Plot STRUCTURE Assingment
STRUCTURE Assingment
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
S.c El Lag.
S.c Baltra Rd
S.p Baltra Rd.
S.c x S.p Baltra Rd.
S.p Garrap.
Mean
±0.95 Conf. Interval